

Scientific Freedom and Hunger Strikes

SUNDAY, June 10, 1973, marks a sad day indeed in the history of the Soviet Union. On that day seven scientists, all Soviet citizens, started a hunger strike designed to bring attention to the fate of scientists who, in the recent past, have attempted fruitlessly to obtain permission to emigrate to Israel. Whether this situation is any worse now than it was a few years ago cannot really be gauged but the fact that the Soviet Union is now relatively open to communication by telephone makes it easier for the rest of the world to hear of, and appreciate, the conditions in which the Soviet scientific profession works.

One of the latest sufferers of injustice is Professor Mark Azbel who was, until recently, head of the Department of Electron Theory of Metals at the Landau Institute of Theoretical Physics in Moscow. Professor Azbel's treatment at the hands of Soviet officialdom is so similar to treatment meted out in the past to other members of the Soviet scientific community that it is only the name that needs to be changed in accounts which *Nature* has published more than once in recent years. What is sad in the case of Professor Azbel, however, is the fact that he and his wife have been separated from their 12 year old son, a point brought out most poignantly in a letter from him dated June 8, addressed to "Scientists of the World":

"For 20 years I was engaged in science in the Soviet Union. I want to live the rest of my life in Israel. My 12 year old son is already there. He got his permission on March 27, and yet I am not allowed to leave the USSR. I am not accused, as many others are, of being involved in the secret activities, possibly because of the topics of about 100 of my papers which were published in the scientific magazines all over the world and which were submitted to these magazines from the well-known institute. I happen to be guilty just in being the doctor of science who must not be useful anywhere outside the USSR, and I am condemned to be separated from my son and to be forcibly detained in the USSR.

"I do not know who passed this sentence on me. I do not know for how long it will act. I cannot even appeal against it. I have not got any written sentence. It was told to me only orally and only by the irresponsible visa official, the only secret court of our time who announces the most essential judgement in the life of the Jew who wants to live in Israel—without any law, any lawyers, any defenders, any publicity and even without any written sentence. Will the world accept such a bible of inquisition customs, such absence of any control, such arbitrariness? Are the scientists and their societies so weak as to allow the scientific degree to become the stamp of state property? Will the lawyers and their international organizations be silent? I cannot be silent. I appeal! Scientists over the world help me! I can never get rid of my doctor's thesis. Does it mean I shall never see my son in Israel?"

Not only are the Soviet authorities apparently determined to bring to a halt the career of any scientist who

deigns to apply for permission to emigrate to Israel, but it seems that an element of the treatment is to disrupt families and to bring personal unhappiness into their lives. It is only two weeks since *Nature* (243, 313; 1973) highlighted the unfortunate case of Dr Evgeny Levich who was whisked away from his family and, as far as can be ascertained, is still at Tiksi in the Arctic, carrying out a period of military service. Now Professor Mark Azbel and his wife are faced with the agonizing decision of whether to leave their son in Israel or to attempt to reunite the family in the Soviet Union which, in all probability, would put an end to all further thoughts of emigration.

But what do the seven Soviet scientists hope to achieve with their hunger strike? By their own words they intend to prove that they belong to nobody but themselves. They state that the Soviet Union claims them as its own property and so "As a matter of principle and provided all other ways are exhausted, [we aim] to destroy this property rather than recognize that anybody is entitled to our souls and bodies". They will end their hunger strike, according to reports, if they are granted exit visas. But it must be asked if this hope is not fulfilled whether in fact there are no other ways of helping these men. The scientific community would suffer a grievous loss if they became martyrs for this cause. This is not to say that the importance of their campaign should be underestimated, but brave men who are prepared to die for their beliefs will do their cause more good if they stay alive to fight.

100 Years Ago



As a supplement to the extract from James Forbes' "Oriental Memoirs," given by Dr. Gulliver in *NATURE* (vol. viii. page 103), the following incident, recorded by Capt. Johnson, deserves republication:—

"I was one of a party at Jeekarry, in the Bahar district; our tents were pitched in a large mango garden, and our horses were picqueted in the same garden at a little distance off. When we were at dinner, a Syce came to us complaining that some of the horses had broken loose in consequence of being frightened by monkeys (*i.e. Macacus Rhesus*) on the trees. . . . As soon as dinner was over, I went out with my gun to drive them off, and I fired with small shot at one of them, which instantly ran down to the lowest branch of the tree, as if he were going to fly at me, stopped suddenly, and coolly put his paw to the part wounded, covered with blood, and held it out for me to see. I was so much hurt at the time that it has left an impression never to be effaced, and I have never since fired a gun at any of the tribe.

G. J. R.

from *Nature* 8, 163; June 26, 1873



NASA's Shuttle Shuffle Endangers Space Science

EVEN before its first flight, the space shuttle has demonstrated a remarkable capacity for dodging hostile fire. Some 18 months ago, in response to bitter opposition from the Office of Management and Budget over the costs of the shuttle, NASA officials radically altered its design, choosing a model which will be only partially recoverable in place of the fully manned, reusable launch vehicle that was originally on the drawing board. The switch cut the estimated development costs in half, secured support from OMB and easily won approval from Congress in a key vote which allowed NASA to let contracts for the project. Undaunted, however, the shuttle's critics in the Senate have continued to attack NASA's economic analysis of shuttle operations in the hope that they can shoot enough holes in it to persuade Congress to delete funding for the project when NASA's budget comes up for approval. But they have found that one of their key weapons—a critical report prepared by the General Accounting Office—has been badly blunted by yet another abrupt change of tack by NASA (see *Nature*, **243**, 372; 1973).

In short, NASA was claiming last year that use of the shuttle will save some \$5,200 million between now and 1990 when compared with the costs of a comparable space programme using conventional, expendable launchers. But the GAO was about to publish a report criticizing NASA's figures and suggesting that cost overruns and other uncertainties may reduce the claimed economic advantages of the shuttle, when NASA came up with a completely fresh analysis of shuttle operations which, it is claimed, will save as much as \$16,000 million. The agency has yet to make a formal announcement of its new mission model, and it has yet to provide details of how those remarkable savings are to be made, but according to one NASA official, the chief change from previous plans is the idea of using the shuttle for sortie missions with the spacelab which may be developed by ESRO. The spacelab will cut the cost of payload development and thereby allow NASA to make more shuttle flights. In that case, it is not difficult to see why NASA is anxious that ESRO should develop the spacelab.

But, as important as costs are to members of Congress, this complex debate over constantly shifting economic analyses to some extent misses the most important question about the shuttle: is it the best vehicle for carrying out a productive and useful space programme? So far, NASA's promotion of the space shuttle has been a clear case of putting the cart before the horse. Instead of first choosing what objectives should be accomplished in space in the 1980s, and then planning a launcher system to fit in with those objectives, NASA has sold Congress the concept of the shuttle and is now discussing what it will be used for. It has, for example, asked the Space Science Board of the National Academy of Sciences to offer advice on how the shuttle can best be used for scientific missions. It goes without saying that such a study should have been made two years ago, and it is not sufficient to argue that political realities forced NASA first to sell the concept, and then come up with the justification.

There have been signs, however, that critics of the shuttle in the Senate have changed their line of attack, and are asking some embarrassing questions about the effect of the shuttle on other space activities in the next few years. Their questions deserve a better answer than NASA has so far given. In short, they are concerned that the projected costs of developing the shuttle will squeeze out more valuable and productive programmes.

That concern has gained considerable momentum from a table supplied by NASA to Senator James Abourezk which outlines the runout costs of all the programmes contained in NASA's budget request for 1974 (in other words, the table does not include any estimates for new projects). The table shows costs of the shuttle increasing from \$200 million last year, to \$1,100 million in 1976 and \$1,190 million in 1977, while the run-out costs of space science and applications programmes decline steadily from \$868 million last year to \$422 million in 1977.

The worrying thing about the table is that although it takes no account of any possible new programmes, it shows no decline in NASA's total spending until after 1977. The clear implication is that unless NASA is able to persuade the Office of Management and Budget and Congress of the need for a larger total budget, there will be no money for new projects until after spending on the shuttle peaks in 1977. The consequences of such a hiatus are surely sufficiently alarming to require an explanation from NASA.

But the standard response from NASA officials is that they are sure that the agency's total budget will soon get back to the level of two years ago—about \$3,400 million. But the cold fact is that this year the Office of Management and Budget trimmed NASA's budget request to \$3,100 million. Spending projections for next year suggest a total budget of \$3,160 million and there is absolutely no guarantee that it will pick up again.

What then would be the consequences if NASA's optimism turns out to be unjustified, and there is no money for new programmes for the next few years? The High Energy Astronomy Observatory has already been suspended, and its missions considerably reduced in scope. The Grand Tour of the Outer Planets has gone by the board, as have several of the Apollo missions that were expected to be most productive and two Orbiting Solar Observatories have been scrapped. Those projects were dropped partly because of budgetary pressures in the past few years, but also because NASA would not be able to support them and the shuttle without a large budget increase. In a sense, therefore, the shuttle has already cut deeply into the space science programme, and it will cut even more deeply if there is no money available for the Venus Pioneer mission, which will be up for funding next year, or even for the revamped HEAO missions. NASA's nimble footwork in dodging criticisms of the shuttle has thus secured approval for the project, but it has also seriously endangered the health of space science for the next few years.

OLD WORLD

Trouble still Brewing over Law of the Sea

BRITISH proposals affecting the law of the sea have been heavily criticized by the Parliamentary Group for World Government. The all party group, which embraces both houses of Parliament, declared last week that the proposals would "effectively remove from mankind the richest part of its heritage, endanger freedom of scientific research and increase the likelihood of international conflict".

The proposals that have aroused the group's fury provide for a grid system—of the type used to regulate oil prospecting in the North Sea and elsewhere—which will divide up the area of the world's ocean into administrative areas for exploitation.

The proposal has been about for some time, but the group's action appears to be prompted by the start, on July 2, of the final preparatory meeting for the United Nations' Law of the Sea Conference planned for Santiago next year.

Under the British proposal—which is by no means guaranteed approval by the Santiago conference—the world's oceans would be divided up into large rectangles which any nation could license from the International Sea Bed Authority that is to be set up by the Santiago conference. Each nation could then exploit its part of the oceans. The proposal also provides that only a small percentage of the oceans would be licensed initially—perhaps five or ten per cent—in order that the developed nations do not gain further economic advantages over the under developed.

To further ensure what is described as an "equitable distribution", the licences would be granted on a quota basis, which takes into account population.

Developing countries would also be able to sub-licence companies in developed countries to actually exploit sea bed resources for them, benefiting both from the immediate return on the work and from the experience of the technology.

The parliamentary group ridicules this scheme describing it as a twentieth century enclosure act and predicting that it will cause international conflict. Unfortunately the group has nothing to put in place of the British proposal.

There is, however, an alternative, and currently it seems to have more explicit support than the British proposal. Its advocates include many of the developing countries and its aim is to create an International Sea Bed Authority that will actually exploit the oceans itself, distributing the profits after administration costs.

Objections to the scheme—largely

voiced by the developed countries—are that the cost of setting up such a commercial organization to exploit the minerals and hydrocarbons beneath the sea-floor would be astronomical. Sums larger than the entire budget of the United Nations would be needed, Britain has claimed.

Defining the constitution of such an authority might well prove an insuperable task, as it would have not only to be strong commercially, but would have to be able to resist political pressure—for example from copper producing countries who might try and restrict the mining of manganese nodules. Equally, past experience with international organizations does not suggest that a commercially orientated one would be any more successful than its diplomatic predecessors.

The parliamentary group also warns of the dangers presented to scientific research by other proposals before the conference. Extension of "patrimonial seas" to 200 miles from land—a proposal that may well be approved in one form or another at Santiago—could hamper scientific research, the group claims, and in doing so it is reflecting well-founded

fears that the scientific community has been expressing for some time (see *Nature*, 239, 421; 1972). A large block of the developing countries, notably the South Americans and Africans, are claiming that scientific research is merely a mask for commercial exploitation of the seabed (in the words of a Peruvian delegate "knowledge is power and must therefore be controlled"). What the developing countries are proposing is that scientific research should be carefully regulated by the International Seabed Authority, which would not only grant permission for scientific research on the high seas, but which would also have access to all results before they were published.

Currently this proposal is being strongly opposed by the developed countries—USSR included. Firm declarations have been made that scientific research must be kept free. It remains to be seen how much the attitude of the Africans and South Americans is simply a negotiating position, but there remains a danger that scientific research could suffer if the interests of defence and economics alone rule in Santiago next year.

NATURAL RESERVES

Uranium Found in Britain

SEVERAL thousand tons of uranium oxide ore have been found in Britain. The discovery of the ore was in fact made some years ago at the start of the Institute of Geological Science's five year search for uranium which it has been conducting on behalf of the United Kingdom Atomic Energy Authority. The £0.25 million programme revealed deposits of the ore in northern Scotland, Cornwall and Devon, but full announcement of the discovery was not made until last week. A preliminary announcement that some ore was present near Ousdale burn in Caithness was made in 1970¹.

IGS is unwilling as yet to say how much ore has been found, or to say precisely where it is, although, apparently, it is not located in national parks. Dr S. H. U. Bowie, chief geochemist at IGS, said this week that while the ore is not of grade one standard, it still represents an "important strategic reserve".

The current price of recovered uranium oxide is just under \$10 a pound. The price will have to rise to between \$10 and \$15 a pound before

it becomes economic to mine the newly discovered deposits. At present there is a world surplus of uranium but by the mid-1980s it is expected that the price—which has remained stable for some time—will begin to rise. At that point the British deposits might prove worth mining.

Although they are small in comparison with the estimated world deposits (100 million metric tons, 2.5 million million of which are within a mile of the surface), they might, if they proved to be in the order of 8,000 tons, provide enough energy to meet the equivalent of the whole of the United Kingdom's current electricity demand for about 50 years. Whether in fact the find will be good for that long will not be known until the IGS provides more details, probably later this year.

The five year programme, which ended in March, involved searching five areas of Britain—northern Scotland, the southern uplands of Scotland, the Midlands, Central Wales and south western England. Before the search began the likeliest sites were considered to be Devon and Cornwall and northern Scotland, including the Caithness and Orkney areas.

¹ Institution of Mining and Metallurgy Transactions B, 79, 180; 1970.

RESEARCH AND DEVELOPMENT

Cambridge Science Park

CAMBRIDGE is shortly to open a science park. By the autumn the first light industry concentrating on technology advanced products that require a high degree of research and development will be installed on a site to the north of Cambridge.

The scheme, sponsored by Trinity College, emerged after a university committee under Sir Nevill Mott had recommended, in 1969, that science based industry in Cambridge should be expanded.

The report was well received, and Trinity, largely because it had some derelict land to spare, sufficient capital and an enthusiastic bursar who is also a scientist, took on the task of planning and building the park.

When it is completed the 13.5 acre site could house more than 10 companies, although initially Dr John Bradfield, Trinity's bursar, hopes to see about six companies move in. The first of these will be Laser-Scan. Set up by a group of physicists from the Cavendish laboratory, the company specializes in designing and building computer guided laser beams for counting nuclear particles. Other types of industry that Dr Bradfield hopes will be attracted to Cambridge are, for example, companies working on amino acid sequences, thin metal films and any similar science based work that requires intensive research and development.

The object of the science park is a two way exchange of ideas and personnel. Research students, the theory runs, will benefit from contact with high technology industry and the industry will benefit from having some of the best research facilities in the country at hand. Professors will be able to keep in touch by acting as consultants, and Cambridge may gain up to a thousand jobs.

The road to the park's opening has not been entirely smooth, however. The town had to be talked into accepting a development that went against its declared plans, and the college had to cope with rising building costs, but now that the first buildings are going up and service facilities are being put in, Dr Bradfield is beginning to advertise for clients confident that the scheme is more than an expensive dream.

The park hopes to attract two types of client. One will be the small, new company, specializing in a scientific field, and the other will be the highly specialized research department of large companies or public utilities. Once a company moves into anything resembling large scale production, it will have to move off the park.

The Cambridge Science Park is not

unique in Britain. Other universities have close ties to science industries. But it is one of the rare deliberate attempts to create an interchange of research and production experience between universities and industry. In the United States, there are over 80 science parks, one of them more than 3,000 acres in size, but 27 of them are wholly limited to science-based industry of the type that Cambridge wants to attract. Britain offers far fewer opportunities for research ideas to be transferred directly into products.

So far, Trinity has put £200,000 into the scheme, but Dr Bradfield insists that it is not intended to be a profit making operation. "We hope it will cover its costs in the foreseeable future," he says, "but there are no plans to make a profit. It is after all an academic service."

MEDICINE

Rationalization Delayed

THE University of London's plans for the reorganization of undergraduate teaching in medicine and the biological sciences are in danger of being thwarted by the University Grants Committee. Recently the UGC turned back to the university its list of priorities in this field and the university was asked to reconsider its plans.

Following the publication of the Todd Report in 1968, which recommended that the teaching of medical students and that of biological science undergraduates should be more closely linked, the university after a long period of gestation decided on three schemes to implement these recommendations. The first plan is to place the pre-clinical and clinical medical schools of St Thomas's Hospital, the clinical medical school of Westminster Hospital and the School of Biological Sciences of King's College at one site. The second is to similarly site the pre-clinical parts of the medical schools of the London Hospital and St Bartholomew's Hospital contiguously with the biological sciences department of Queen Mary College. The third scheme is to house the pre-clinical departments of the Royal Free Hospital medical school and of the University College Hospital Medical School and the faculty of medical sciences of University College at a common site.

The University of London in its application to the UGC placed the St Thomas's, Westminster and King's scheme at the top of the list but the University Grants Committee clearly did not favour giving first priority to the most expensive scheme—which is estimated to cost about £6 million—and the university has been asked to review its priorities. A spokesman for the St Thomas's, Westminster and King's

scheme, said this week that he feared that this might mean that this scheme would now be given a low priority although he felt that there were compelling reasons why this should not be so.

One of the difficulties with this scheme has been the choice of site. After consideration of possible sites both in Covent Garden and on the South Bank, a site at St Thomas's Hospital was finally chosen but this has run into difficulties recently. Contrary to first expectations the site is not the property of St Thomas's Medical School but it is owned by the Department of Health and Social Services. Negotiations are at present taking place over what price, if any, should be paid for the site. A spokesman for the scheme said that the question of evaluating the site should not affect the plans and that it had always been understood that the site would be available.

The other schemes have not had a clear passage either and letters to the press a year or so ago made it clear that the staff at St Bartholomew's Hospital did not favour moving from their present site.

The next move is up to the University of London which has to reply to the UGC's request for a re-evaluation of the scheme.

Whether or not work starts on the proposed centre at St Thomas's Hospital on schedule depends to a great extent on the University's reply.

The present plan is that if the money is granted soon, construction can start in 1975-76 and that the first students can be admitted in the autumn of 1979.

CONSERVATION

Friends of the Whales

MONDAY sees the start of this year's meeting of the International Whaling Commission. Friends of the Earth and a whole host of other conservationist organizations are again attempting to pressure the commission into imposing a ten year moratorium. Advertisements in *The Times* next week, and letters signed by conservation organizations world wide and despatched to the heads of state of the commission's member countries are all aimed at achieving the moratorium which was approved by the Stockholm Conference 53 votes to 0 last June.

But the chances of a moratorium are no greater this year than last. Last year's meeting, small though the progress it made was, did at least move the commission some way down the road that the conservationists want it to go, and a further step this year in the form of protecting certain species further and cutting quotas again, could effectively outmanoeuvre the moratorium bid.

NEW WORLD

Double Trouble for the AEC

by our Washington Correspondent

THE Court of Appeals last week set the Atomic Energy Commission back on its heels by ordering it to produce an analysis of the environmental effects of the entire fast breeder reactor programme. It was a landmark decision in many respects, for it gives opponents of the breeder a powerful tool for opening up public debate about alternative methods of producing energy in the 1990s, and it also has important implications for other high technology ventures such as the AEC's Plowshare Programme. But that was only the first of the commission's headaches last week, for the court decision was followed swiftly by the resignation of Mr Milton Shaw, who for the past nine years has been head of the AEC's reactor development programme and one of the most powerful officials in the agency. The two events were not entirely unconnected, and it remains to be seen which will turn out to be the more important.

The court decision was the final move in a case filed against the AEC last year by the Scientists' Institute for Public Information (SIPI), a group of scientists headed by Barry Commoner and Margaret Mead. At issue is the development and possible proliferation of the liquid metal fast breeder reactor (LMFBR), the Administration's chief technological fix for the energy crisis and a prime target for criticism from environmentalist organizations.

The AEC, in conjunction with the Tennessee Valley Authority and private industry, is building a prototype LMFBR at Oak Ridge in Tennessee at a cost of some \$2,000 million. The goal is to have a successful demonstration of the reactor by 1980 and to prove that it will be commercially attractive. After that demonstration, private industry will take over the reins and the AEC is projecting that by the year 2000 LMFBRs will be producing as much electricity as all the present power stations put together.

SIPI's law suit revolved around the question of whether the commission is obliged by law — specifically, the National Environmental Policy Act (NEPA)—to produce an environmental impact statement only for the pilot LMFBR, or for the commercial development that may follow. The AEC has already produced an impact statement for the pilot plant, but maintained that it would have to gaze into the crystal ball to produce an analysis of the

environmental implications of the entire programme. Such an analysis, it said, "would be meaningless in terms of content".

In November last year, a lower court judge agreed with the AEC and threw the suit out, but SIPI went to the Court of Appeals where it won its case last week. The appeals court opinion, written by Judge J. Skelley Wright (who also wrote the opinion in the so-called Calvert Cliffs case which forced the AEC to consider environmental factors

during the licensing of nuclear reactors), accused the commission of taking "an unnecessarily crabbed approach to NEPA". His opinion also throws up some interesting points about the development of projects involving advanced technology.

Judge Wright argues that if the AEC waits until the breeder reactor is on the verge of commercial application before it files a statement on the environmental impact of the project, so much money would be invested in the technology that

HUMAN EXPERIMENTATION

NEW Policies Criticized

by our Washington Correspondent

THE federal government's policies for protecting the rights of those who take part in medical experiments came under fire last week in a report made public by the Department of Health, Education and Welfare (HEW). Prepared by a special ad-hoc committee set up to investigate the notorious Tuskegee Syphilis Study—a study of the effects of untreated syphilis among poor black men in Alabama—the report terms the policies vague and overly general, and suggests sweeping reforms in the system of regulating human experimentation.

Even within HEW, the committee notes that there is no uniform policy for the protection of research subjects, and points out that there is also no uniformity among other agencies of the federal government which support human experimentation. "The lack of uniformity", the committee states, "creates confusion and denies some subjects the protection they deserve".

At present, HEW relies heavily on review committees in universities and other research institutions for preserving the rights of research subjects, and the department recently issued a set of guidelines for the review committees to follow. Reliance on the committees "was an important advance", the report suggests, but the system suffers from basic defects which seriously undermine its effectiveness. In particular, the HEW guidelines are vague, have little to say about research involving children, prisoners and other groups where special considerations apply, and there are no effective procedures for enforcing them.

Accordingly, the committee suggests that Congress should establish a permanent body independent of any exist-

ing federal agency, with authority to regulate at least all federally supported research involving human subjects. The idea is that it would consist of a board, composed of people from diverse scientific disciplines and representatives from other professions, which would formulate research policies in much greater detail than is now the case, and draw up procedures to make sure that the policies are implemented by institutional review committees. The board, which the report suggests could be called the National Human Investigation Board (NHIB), would also establish appeals procedures for adjudicating disagreements between investigators and institutional review committees.

In research institutions themselves, the committee suggests that an Institutional Human Investigation Committee (IHIC) should be established as a direct link between the institution and the NHIB, and that it should have two distinct subcommittees: a protocol review group concerned with prior review of research protocols, and a subject advisory group to aid subjects with any problems concerned with their participation in the research. In particular, the subject advisory group should be concerned with procedures for making sure that a subject's consent to take part in a study is freely given after full information about the research has been provided to him.

Senator Edward Kennedy recently held a set of hearings with his Senate Health Subcommittee on the problems involved in human experimentation, during which a number of particularly glaring examples of dubious ethical practices were brought to light. He can be expected to introduce legislation soon to strengthen regulations designed to protect the rights of human subjects.

(Final Report of the Tuskegee Syphilis Study Ad-hoc Advisory Committee, available from the Center for Disease Control, Atlanta, Georgia.)

proper balancing of considerations will be difficult. In short, "technological advances are . . . capital investments and, as such, once brought to a stage of commercial feasibility, the investment in their development acts to compel their application". And, perhaps more important, by the time the breeder has reached the point of commercial feasibility, other options would be foreclosed.

Critics of the breeder reactor have long maintained that the Administration is sinking money into the project at the expense of other approaches, such as geothermal energy, solar power or controlled thermonuclear fusion.

They will thus use the court decision to stimulate a public debate about such alternative sources of energy, for NEPA specifies that environmental impact statements must consider alternative programmes.

Shortly after the court decision was handed down, Dr Dixie Lee Ray, the new AEC chairman, announced that it would not be appealed to the Supreme Court. The commission expects, however, that it will take several months to prepare the impact statement.

The court's decision has other implications outside the breeder reactor programme, and it could well prove to be one more nail in the coffin of the Plowshare Programme. Judge Wright's opinion essentially states that environmental impact statements for entire programmes must be written at the pilot plant stage. It thus seems to imply that if the AEC intends to follow up the recent Rio Blanco explosion, which was designed to test the feasibility of using nuclear explosions to extract natural gas in deep deposits in Colorado (see *Nature*, **241**, 494; 1973), it will have to outline the possible environmental consequences of complete commercial exploitation of the gas deposits. That would entail several thousand explosions, and opponents of the project are confident that when all the details are spelled out they will create devastating opposition.

As for the resignation of Mr Shaw, he is known to have been considerably upset by a recent reshuffle of the AEC bureaucracy which removed safety research and development from his jurisdiction and placed it in a division on its own (see *Nature*, **243**, 182; 1973). The decision to comply with the appeals court ruling instead of taking it to the Supreme Court was reported to be the last straw, and he decided to quit.

A powerful administrator who has incurred the wrath of the AEC's critics, Shaw has been accused in the past of paying insufficient attention to research that casts doubts on reactor safety. He has ruled the division of reactor development with an iron hand, and in so doing has won the approval of the Joint Committee on Atomic Energy because of his powerful influence in the development of nuclear power. His departure,

coupled with the new arrangements for safety research, may help to assuage some of the fears of the critics of nuclear power.

In moving safety research out of Shaw's division and also in deciding not to contest the appeals court decision, Dr Ray has considerably strengthened her control over the agency. She has also established her independence from the Joint Committee—which has consistently supported development of nuclear power—and incurred the displeasure of some key members, notably Chet Hollifield, who are upset about the recent events.

NUTRITION

Protein Shortage

from a Correspondent

THE Protein Advisory Group of the United Nations System called for urgent action to meet the growing world shortage of protein at its twenty-first meeting held in New York earlier this month. The group recommended:

- The implementation of the coordinated national food reserve policies proposed by FAO.
- A global approach to world fishery management.
- Research on enhancing animal productivity and improving the yield of legumes.
- Developed countries to provide additional assistance to developing countries to help expand food production, by the provision of fertilizers, plant, equipment, etc.
- Substitution of vegetable proteins for animal proteins by, for instance, the utilization of meat-like analogues and milk substitutes.
- The development by governments of food distribution systems which ensure the supply of basic food to meet the needs of vulnerable groups.

The group's recommendations came after Mr Lester Brown of the Overseas Development Council, Washington DC, had pointed out that historically the global food situation has been largely discussed as a population and food supply problem. Now, however, rising affluence is a new claimant on world food resources. Four-fifths of the annual increase in world population occurs in poor countries, which are short of employment as well as food; in these countries the *per capita* availability of grain averages about 190 kilograms a year, most of which is consumed by people. In contrast almost one ton of grain is consumed per head of the population of the United States and Canada, but only 70 kilograms are consumed by people, the rest being used for animal production.

AIR POLLUTION

Setback for the EPA

THE Supreme Court last week ruled that much of the Environmental Protection Agency's strategy for cleaning up air pollution is illegal. The ruling, which is certain to lead to more court action, instructs the EPA to prevent state governments from allowing air quality to deteriorate significantly below present levels. It means that the agency must alter its approach to curbing air pollution.

The basis of the court case is the Clean Air Act, which lays down specific air quality standards that must be met across the United States. The EPA had approved plans drawn up by state governments for complying with the act, which allow the air to deteriorate in quality in regions where the standards are already being met, provided that it continues to stay below those standards. But the Sierra Club last year took the EPA to court, arguing that the Clean Air Act does not allow deterioration of present air quality. It won its case in a lower court in June last year, it was upheld by a court of appeals, and last week the Supreme Court was evenly divided on the issue, which has the effect of affirming the lower court ruling.

The EPA, backed by several industrial and commercial groups and some state governments, argued that unless some deterioration is allowed in air quality, there can be no industrial or population expansion in areas which have pristine air, and there also can be no relocation of industry outside the smog-ridden cities.

There is, however, some leeway in complying with the court decision, because the lower court prohibits only "significant" deterioration in air quality, without specifying exactly what significant means. The EPA is thus considering two alternative approaches. Either it will go ahead and approve state plans which would still allow deterioration, but at a level which the agency considers insignificant. That course would almost certainly lead to fresh court action in areas where environmentalists do not agree with the EPA's definition of significant. Alternatively, the agency is considering agreeing to state plans which specify that sources of pollution should use the best available technology to clean up their output of pollutants.

It seems likely that the agency will adopt the second approach, in which case it may have a difficult time arguing in court that it is preventing significant deterioration, and instead of fighting over a few areas, it will again be involved in litigation over the entire cleanup effort. It will keep the courts busy.

World Health Organization and Genetic Disorders

This critique of the World Health Organization report number 497 on *Genetic Disorders: Prevention, Treatment and Rehabilitation* is by Professor J. H. Edwards, of the Department of Human Genetics at the University of Birmingham

A COMMITTEE of cobblers, if asked to advise on the health of the world, would doubtless draw attention to the scandal of millions of naked feet and to the danger of foot-borne diseases, and advise on the need for counselling of the barefooted and for the training of both counsellors and cobblers.

This form of negative stability is by no means peculiar to health; bee-keepers, chess-players, numismatists and pot-holers share an equal missionary zeal. Even solipsists, according to Bertrand Russell, complain of their rarity.

Medical geneticists, whose report was reviewed briefly in *Nature* (238, 7; 1972), might be thought to be free from this hazard by their training in the biases of ascertainment, but this is not so.

The report makes a number of recommendations which are neither surprising nor inappropriate. For reasons which are not clear, however, it contains a strange mixture of doubtful assumptions, erroneous facts, and uncertain inferences, some of which overflow into recommendations. The first paragraph speaks of "the relative increase in morbidity and mortality attributable to slow changes in gene frequencies". No explanation is given. There is said to be "no reliable information on the current prevalence of genetic disease"—in fact, the information is adequate for practical purposes; the numbers of the deaf, the blind, and the ineducable are necessarily known in all countries with compulsory education, and the proportions of these due to mendelian and chromosomal disorders have been estimated on good data.

The word genic is extended from its regular use to include multifactorially determined disorders, a term used variously. In the table on multifactorial disorders, congenital malformations and conditions of unknown and "non-genetic" disease are used as exclusive categories. In Table 2 a series of malformations, mostly of unknown aetiology, are called multifactorial, which admittedly means the same thing.

Later we are redeemed with robust and orderly descriptions of biochemistry

until we again leave the ground in a cloud of numerals. On page 31 a mutation rate for recessives of 10^{-4} is "assumed". On the next page $100 \times 5 \times 10^{-5} \times 2 + 0.0001$ somehow makes 0.0086. On page 33, "genetic counselling, urging couples total abstinence from reproduction" is recommended in recessive traits, but, on page 34, we are almost all carriers—"every zygote is estimated to carry 1 or 2 deleterious recessive genes".

On page 43 the remarkable claim that fibrocystic disease can be diagnosed *in utero* is made. The claim that sickle cell disease can be diagnosed, which is also made, was hardly stronger when the committee met. On page 45 compound interest is used to provide the doubling time for "multifactorial disorders". The theory is difficult and no mention of the procedure is made, but certainly stabilizing mechanisms are likely to reduce this. The report ends on a most alarming graph, whose credentials are hardly as firm as the chronology of Archbishop Ussher, showing that the end of the world is at hand.

How, we may ask, can such an assembly of experts, with a dedicated and able secretariat, lead to the publication of a document so rich in platitude and inconsistency?

The reason is not far to seek: an assembly of experts, appropriately stratified by hemisphere, power-block and language, is expected to create, within one week, an agreed text on a subject beside which Vietnam and the Common Market are mere tea-cup storms restricted to parts of continents for a mere generation or two.

The problems presented by the genetic future of man are far more important than anything under the control of warriors, bankers, or tradesmen, and, by their nature, no executable advice can be expected from a week's discussion. What seems important is that no one should expect the committees or secretariats to undertake such impossible tasks. Given its magnitude the achievement may well be commended, but the results are not conducive to the orderly development of either practice or theory.

Perhaps the World Health Organization technical report series should restrict itself to technical reports and provide summaries of what is known, linguistic recommendations, and recipes for laboratory procedures; this may seem a rather pedestrian approach, but, in a world short of bread, elaborate schemes for cake-making should be thoroughly tested on the rich for acceptability and digestibility before being offered to the poor.

Technical reports should surely be reports of technical matters. By tackling some specific problem, such as G6PD, clotting factors, or immunoglobulins, this series has had a profound influence on the standards of human genetics throughout the world and the informal benefits of meetings of world experts have been accompanied by formal reports which have defined problems, increased the precision of words, and provided a framework from which local policy decisions could be made.

The recent report on haemoglobins shows once again the benefits which a central organization can confer simply by assembling experts and reviewing facts.

Recommendations

THE WHO Scientific Group on Genetic Disorders met in Geneva from November 16 to 22, 1971. Its chief recommendations included the following:

- medical genetics services, including counselling clinics, should be provided at medical centres;
- physicians and the public should be better educated in the principles of genetics and their relevance to human welfare;
- better facilities for prenatal diagnosis should be developed;
- more research should be undertaken, particularly in certain areas, including prenatal diagnosis, population screening for heterozygous carriers of specific mutant genes, and the identification of environmental pollutants that might increase mutagenesis. Follow-up studies on children born after amniocentesis are also needed to assess the long term effects of the procedure, as are studies on the effects of inborn errors of metabolism in the mother or the fetus;
- medical genetic centres should set up registries of genetically determined disorders.

NEWS AND VIEWS

Marginal Basins Form by Seafloor Spreading

THE problem of the origin of the marginal basins which lie between trench-island arc systems and their corresponding continental margins has vexed Earth scientists for as long as any living geologist can remember. Of course, there has been no shortage of proposals, either for the origin of marginal basins in the context of island arc development as a whole or for the formation of single basins seen as localized phenomena. In the early part of this century, for example, the discovery within island arc systems of rocks thought to be characteristic of continents led many geologists to believe that marginal basins were continental areas which had subsided, subsidence being attributed variously to crustal warping associated with orogenic compression, subcrustal erosion, oceanization (and that very recently), and many other processes. On the other hand, Wegener, who quite naturally viewed the world in terms of continental drift, saw marginal basins as the extensional spaces left in the wake of moving land masses.

Not surprisingly, the conflict between the various hypotheses for the origin of marginal basins stood little chance of resolution until much more information about the basins themselves became available; and the acquisition of hard data only became possible with the development of reliable marine geophysical techniques during the late 1950s and early 1960s. Then, for example, seismic refraction studies showed the crust beneath marginal basins to be similar to that of normal ocean basins and bathymetry indicated that the basins have oceanic depths which are intermediate to normal. These and other discoveries clearly required the rejection of all hypotheses describing marginal basins as essentially continental; and with the field thus narrowed the way was open to a more detailed comparison between marginal basins and the wider ocean floor the origin and evolution of which were coming to be seen in terms of the now-familiar processes embodied in the new global tectonics.

The next important step was the deduction from a wide variety of geological and geophysical evidence that marginal basins must be created by crustal extension. Apparently it took some time for this conclusion to follow from the available data, for as late as 1969 some workers were attempting to explain the high heat flow in some marginal basins by models which did not involve extension. But in an article which has now become an important landmark in the subject, Karig (*J. geophys. Res.*, **76**, 2542; 1971) convincingly pressed the case for crustal extension from a consideration of all geological and geophysical data then available. In the process, he found it convenient to divide marginal basins into two natural groups—the first involving those basins in which crustal extension is currently active (Tonga, Kermadec, Mariana, Bonin and New Hebrides) and the second comprising those in which extension has ceased (the rest). The inactive marginal basins were then further divided into those with high heat flow and those with normal heat flow, the difference being essentially one of age. Karig supposed that once activity in a basin had ceased the crust would cool, so that, by implication, the basins with high heat flow should be younger.

It follows from this view that marginal basins differ principally only in their stage in history, although Karig admitted that the case for extension in the now inactive basins is based primarily on similarities with active basins rather than the result of direct evidence. But accepting an extensional origin for all basins, Karig went on to propose a model for the extension which involved a thermal diapir of mantle material rising buoyantly from the shear-heated upper surface of the downwelling lithosphere. At the same time, Packham and Falvey (*Tectonophysics*, **11**, 79; 1971) were proposing the injection of mantle material close to island arcs, resulting in a form of asymmetric seafloor spreading. Unfortunately, neither Karig nor Packham and Falvey had access to one vital piece of information—an adequate set of magnetic data from a marginal basin.

As Hayes and Ringis show on page 454 of this issue of *Nature*, such data are now available for the Tasman Sea (inactive and with normal heat flow in Karig's classification) and indicate symmetric magnetic anomalies which correspond to part of the global pattern of Cretaceous lineations. In short, the magnetic evidence together with seismic and bathymetric data demonstrate convincingly that the Central Tasman Sea formed between 80 and 60 million years ago by a process of symmetric seafloor spreading almost identical to that now taking place in the principal oceans of the world. In view of Karig's case for the similarity of marginal basins, even this one example strongly suggests that most, if not all, other marginal basins will ultimately be found to have formed in like manner.

P. J. S.

Selecting Sequences

READERS will recall the experiments on evolution in the test tube in which the template dependent RNA polymerase from Q β infected *Escherichia coli* replicates Q β RNA which evolves in response to the metabolic challenges provided by Spiegelman and his colleagues. In particular, when the products are selected for rapid growth, the greater part of the original genome—containing genes not immediately concerned with replication *per se*—are jettisoned and a much shorter molecule is produced. This too can evolve in response to changes in the reaction mixture, and it becomes interesting to ask what detailed molecular changes occur.

Mills *et al.* (*Science*, N.Y., **180**, 916; 1973) have now provided the first steps to an answer to this problem by sequencing the 218 residue molecule which they call MDV-1. The methods used in this operation involve several clever dodges worthy of comment. MDV-1 replicates by way of a two stranded intermediate and both the plus and minus strands are copied though apparently with unequal efficiency. It is thus possible to make a mixture of both strands which can be annealed to give a mixture of the plus strand and a 1:1 complex of the plus

and minus strand; these can then be separated by polyacrylamide/gel electrophoresis and studied separately.

First, the plus strand was completely degraded with both ribonucleases T_1 and A and the two sets of oligonucleotides were sequenced. This was helped by the fact that the 2' or 3' terminal phosphate is derived from the α phosphate of the ribosetriphosphate incorporated in the nearest 3' neighbour position. Because it was possible to grow molecules in a medium with just one of the triphosphates labelled in this position with ^{32}P , it was easy to detect the residues in the oligonucleotides with this nucleotide next to them in the 3' position. In this way the nearest 3' neighbours of all bases can be determined. This in itself gave most of the information necessary to sequence the oligonucleotide fragments, and a complete catalogue of the sequenced T_1 and A digest fragments from the plus strand was thus obtained. The method was then applied to the 1:1 complex of both strands and a combined catalogue for both was found. It was then a simple matter to subtract the plus strand catalogue from the combined catalogue to obtain that of the minus strand.

The nearest neighbour information together with partial overlaps between products obtained from the two sorts of enzyme and the fact that the plus and minus strand fragments were complementary enabled the fragments to be assembled into sixty-six extended sequence blocks, and the problem remained to place these blocks in the correct order. The nearest neighbour information severely restricted the possibilities but did not point to a unique solution. To eliminate ambiguities it was necessary to obtain information concerning the order in which the blocks occurred along the chain. This was accomplished by exploiting the ability of polyacrylamide gels to resolve RNA according to molecular weight. The replication system was set up to produce only minus strands and aliquots were removed and replication quenched at different times after initiation. This resulted in a set of partially completed molecules each of which started at the 5' end. These were pooled and resolved on polyacrylamide gels. The resulting distribution was divided into twenty-two fractions and each was

printed. It was thus possible to test each fraction for the presence or absence of relevant enzymatic digestion products. If an oligonucleotide was absent it was clear that it must occur further down the chain than the point at which the RNA in that particular fraction was terminated. In this way it was possible to determine the order of the extended sequence blocks along the chain and to assemble them into a unique sequence.

The payoff of these elegant experiments comes from a close inspection of the final sequence. This was found to contain several complementary runs of nucleotides capable of forming helical regions. These helical regions

tended to occur in positions along the chain which are resistant to enzymatic digestion. The authors point out that if one strand has such potential structure so must the other and that the two strands are, in a sense, mirror images. They also observe that if certain nucleotide sequences are favoured by the selection pressures which produced MDV-1, there would be a tendency for them to exist in both strands. Once this occurs there must necessarily be complementary regions in both chains leading to the possibility of secondary structure. Thus secondary structure in such molecules must be seen as a natural consequence of selection for molecules

Immunochemistry of Hepatitis B Antigen

THE discovery of hepatitis B antigen has provided a valuable laboratory test for potential infectivity of blood and other material, but, in spite of considerable research, the biological nature of the antigen remains unknown. Morphological studies of the antigen by electron microscopy after negative staining revealed a plethora of structures including small spherical 20 nm particles. Some of these particles have several features in common with negatively stained virus particles and the finding of about 5% RNA in partially purified antigen (Jozwiak and colleagues, *Nature new Biol.*, **229**, 92; 1971) suggested a close affinity between the antigen and animal RNA viruses. The failure to confirm so far the presence of RNA in hepatitis B antigen, however, has led to several alternative proposals on the nature of the antigen.

The view that the antigen is composed of normal host-coded material such as serum proteins, assembled through the stimulus provided by infection with hepatitis B virus, and the background of genetic factors has led Blumberg and his colleagues (*J. exp. Med.*, **134**, 3205; 1971) to suggest that the antigen is a unique infectious agent which has the characteristics of an inherited serum protein polymorphism (*Nature new Biol.*, **234**, 226; 1971). Another view is that the antigen is a specific protein or lipoprotein produced by liver cells infected with hepatitis B virus. But the most likely proposition is that the antigen represents excess virus coat material, partly in the form of aggregates of protein subunits, and that the inner core of the 42 nm particle may be the infectious agent.

Biochemical dissection of the antigen now seems to provide further clues. Burrell and his associates report in *Nature New Biology* next Wednesday (June 27) the finding of significant

amounts of carbohydrate in the 20 nm antigen particles. They consider it possible that either the carbohydrate is necessary for maintaining the structure and functional integrity of the antigenic determinants or that the carbohydrate itself constitutes an important antigenic determinant. This raises two intriguing possibilities. First, the carbohydrate might have a novel haptenic specificity which is either virus-coded or virus-induced host cell-coded, or, second, the carbohydrate (and indeed some lipoprotein components) might simply be derived from the host cell membranes as the mature virus particles are released by budding.

The incorporation of host specific antigenic material in the envelope of the virus does not alter the specificity of the viral antigens nor the biological properties of the virions. There may, however, be some antigenic similarities between the virus carbohydrate hapten and the carbohydrate antigens of normal cell surfaces, or between the virus protein and the protein moiety derived from the host, leading to the establishment of partial tolerance because of the antigenic similarity between hepatitis B antigen and "self" antigens. Tolerance may play a part in establishing the persistent carrier state. Another possibility is that antigen specificities which consist partly of host-derived material and partly of virus-derived material may elicit antibodies against such antigen complexes which in turn will react with the host cells, especially with virus infected cells. Such a mechanism may operate in the immunopathogenesis of liver damage in acute viral hepatitis (Farrow *et al.*, *Br. med. J.*, **2**, 693; 1970) and in chronic liver disease associated with hepatitis B infection (Zuckerman, *Immunopathology*, **6**, Schwabe, 1970, p. 436).

containing specialized sequences. The authors see this secondary structure as a phenotypic expression of the primary nucleotide sequence and it may be that the properties of these phenotypes in turn provide extra factors that may play their part in the selection of these evolving molecules. Finally, Mills *et al.* intimate that they have sequenced a mutant of their MDV-1 molecule subjected to different selection pressures.

From a Correspondent

PROTEIN SYNTHESIS

In Praise of Controls

from our Molecular Biology Correspondent

THE history of haemin as a regulator of haemoglobin synthesis has transmogrified itself into an absorbing moral fable. When it was discovered that the output of protein by a rabbit reticulocyte system was heftily stimulated by the addition of a *souçon* of haemin, the scheme of regulation that forthwith leapt unbidden to the mind of molecular biologists, nourished on a lifelong diet of feedback, fell so pleasingly into place in the canon, was so close indeed to the way they would themselves have designed it, as to exclude all doubt of its validity. In such a situation it might have seemed wanton to jeopardize so satisfying a conceit by doing one experiment too many. That experiment has now, alas, been done and in several laboratories at once, and the result is a true Huxleian tragedy — a beautiful and virtuous hypothesis butchered by a gang of ugly facts.

The idea, of course, was that globin, accumulating apace in consequence of stimulation by free haemin, would bind progressively more of this haemin, thereby inhibiting further globin synthesis until new haemin was added to the pool. Mathews, Hunt and Brayley (*Nature new Biol.*, **243**, 230; 1973) have shown, however, that haemin stimulates equally the translation of extraneous messengers in the rabbit reticulocyte system. They have tried this experiment with another globin messenger, from the mouse, with lens crystallin messenger and with a viral RNA. The proteins that were synthesized were fractionated by gel electrophoresis, and were not globin, except when a globin messenger was used, as judged by their electrophoretic mobilities and their autoradiographic tryptic peptide maps.

In the case of EMC virus RNA the control of translation by haemin was less marked, but this is very likely a consequence of reluctant and brief initiation by this messenger and its effect in inhibiting endogenous messenger activity.

The same conclusions were in fact taken a step further by Beuzard, Rodvian and London (*Proc. natn. Acad. Sci. U.S.A.*, **70**, 1022; 1973). In the first place they separated the freshly synthesized proteins from intact reticulocytes by ion-exchange chromatography, and found that haemin engendered essentially the same degree of stimulation on the synthesis of haemoglobin, which makes up the overwhelming bulk of the red cell protein, and that of carbonic anhydrase. They next proceeded to a totally unrelated system, ascites tumour cells, and found that here too the translation of both the endogenous messenger and of added rabbit globin messenger was stimulated by addition of haemin. The effect is not on the initial rate of the synthesis, for the build up of incorporation with time is more or less the same regardless of the presence or concentration of haemin (at least up to a limiting value), but rather on the length of time during which synthesis is sustained, so that the stimulating effect becomes apparent only after some minutes.

Yet another independent demonstration of the lack of specificity of the haemin comes from Lodish (*ibid.*, 1526), whose concern was with the synthesis of the proteins of the red cell membrane, a problem of interest in a number of connexions. The same patterns of amino acid incorporation were found whether the synthesis was observed in whole reticulocytes, or in lysates prepared by cautious procedures, that do not evidently provoke the release of membrane-bound polysomes into the solution. Evidently therefore the membrane proteins synthesized in these cells are not made predominantly on membrane-bound ribosomes (in contrast with extracellular proteins, which are synthesized on membranes for instant export as they come off the assembly line). In fact only three of the fifteen or so principal identifiable membrane proteins, as recognized by electrophoretic analysis, turn out to be significantly labelled, and the labelling patterns indicated that one of them was perhaps a precursor of another. This was confirmed by examination of the tryptic fingerprints of the two products, and by inhibition of the transformation of the one into the other by an active-site reagent specific for serine proteases. Thus only two of the principal membrane proteins are synthesized at the reticulocyte stage, and their molecular weights are estimated by detergent-gel electrophoresis to be about 53,000 and 33,000. The preponderant membrane protein species, therefore, especially the high-molecular-weight spectrins, and the blood-group antigen glycoprotein, are synthesized at an earlier stage of development. As to the effect of haemin, this is just as important for the

synthesis of the membrane proteins as for the globin.

All this does not, of course, deprive the function of haemin in this role of its interest, even though it is presumably not implicated in protein synthesis in most other cells. Mathews *et al.* advert to some observations which indicate that the haemin operates by binding to and inactivating an inhibitor of synthesis. Darnborough *et al.* (*J. mol. Biol.*, **76**, 379; 1973) have, moreover, just come up with some compelling evidence that initiation in the reticulocyte system proceeds in a rather different manner to that of most tacit assumptions. They find that a complex is first formed between formylmethionine-tRNA and 40S ribosomal subunits. This complex then takes up messenger and the 60S subunit, and synthesis now begins. It appears that in the absence of haemin, the concentration of the 40S-tRNA complex runs down rapidly. It seems, therefore, that the mysterious inhibitor intervenes at this point, unless haemin is present. Whether the action of the haemin is purely adventitious is not yet, of course, clear. It is, incidentally, interesting that double stranded RNA, which is a strong inhibitor of initiation of protein synthesis, also according to Darnborough *et al.* interferes with the build up of the tRNA-40S complex. Moreover, Kaempfer and Kaufman (*Proc. natn. Acad. Sci. U.S.A.*, **70**, 1222; 1973) have shown that it works by binding to and inactivating an initiation factor, IF-3 (though their inference is that initiation normally occurs on a base-paired segment of the messenger RNA).

SEED RESEARCH

New Directory

THE publication of a *Directory of Seed Research in Britain* (obtainable from the Scottish Horticultural Research Institute, Invergowrie, Dundee DD2 5DA) should improve communications and reduce unnecessary duplication of research effort. It covers not only agricultural and horticultural crops, but also trees, weeds and wild species. Seed production, processing, quality, storage, dormancy, germination and seedling establishment are among the topics included. The compiler—Dr T. W. Hegarty—has divided the directory into four main sections: (1) an alphabetical list of research establishments; (2) a list of research interests, arranged under name(s) of the individual or group concerned within each institution; (3) a name index; and (4) a subject index.

POLYTENE CHROMOSOMES

Selective Replication

from our Molecular Genetics Correspondent

FORMATION of the giant polytene chromosomes of the salivary glands of *Drosophila* takes place by several successive replications of the diploid genetic material. By the time the final number of nine replications has been achieved, each polytene chromosome has more than one thousand times its usual DNA content, for each successive replication doubles its amount of DNA. But some sequences suffer more doublings than others; a comparison of the amount of DNA in the euchromatic arms of the polytene chromosomes with the DNA content of the chromocentre which is formed by the aggregation of regions of heterochromatin shows that the more condensed heterochromatic DNA does not keep pace with the euchromatic sequences; instead of eight to nine doublings it is replicated at most only a very small number of times.

Prompted by this split in the control of replication in salivary gland cells, Spear and Gall (*Proc. natn. Acad. Sci., U.S.A.*, **70**, 1359; 1973) have examined the replication of the sequences which code for ribosomal RNA. The two hundred or so genes which specify ribosomal RNA are clustered on the sex chromosomes at or close to the site where the nucleolar organizer forms to synthesize ribosomal RNA. This region, although itself replicated during the formation of polytene chromosomes (as shown by incorporation of ^3H -thymidine) and active in the transcription of ribosomal RNA molecules, is surrounded by the heterochromatin which is replicated to a lesser extent and is not transcribed at all.

Control of replication in polytene *Drosophila* cells identifies three types of sequence. In a previous article, Gall, Cohen and Polan (*Chromosoma*, **33**, 319; 1971) used hybridization with satellite DNA to show that these sequences—a major part of the heterochromatin—appear to replicate hardly at all compared with the extensive polytenization of sequences contained within heterochromatin. Spear and Gall now show that ribosomal RNA genes fall into neither of these categories, but are controlled independently.

Cells of *Drosophila* which have the XO constitution (that is an X chromosome and no Y chromosome) have only one nucleolar organizer, whereas female cells of the XX class have two nucleolar organizers. By measuring the extent of hybridization with ^3H -rRNA of DNA extracted from these two types of diploid cell, Spear and Gall found that the content of rDNA is proportional to the number of nucleolar organizers. In one strain of *D. melanogaster* they

found that XO cells have 0.26% and XX cells have 0.47% rDNA; in another strain the XO cells have 0.24% and the XX cells have 0.37% rDNA. This shows that a nucleolar organizer forms at each cluster of rRNA genes on an X chromosome, the two clusters in XX cells presumably being identical.

But very different results were obtained when the DNA used for hybridization was extracted from polytene cells of the salivary gland. In both strains of *D. melanogaster*, only 0.08% of the DNA corresponded to ribosomal RNA. In a polytene cell with the 1,024 equivalents of euchromatic DNA produced by nine doublings of the diploid content, this proportion corresponds to about 150–200 times the amount of ribosomal DNA in a diploid cell. This would be generated by some six or seven doublings of the genes coding for ribosomal RNA.

But the control of replication of the ribosomal RNA genes is not merely set at a lower level than that for euchromatic DNA. In both XO and XX cells, the rDNA content of polytene nuclei corresponded to the same level of 0.08%. The ribosomal RNA genes have therefore doubled one more time in the XO cells to compensate for their lower starting value; the final level of rDNA in these cells is therefore independent of the number of nucleolar organizers with which their diploid ancestors started. In contrast to this behaviour, the remaining sequences of the X

chromosome double only the same fixed number of times in XO cells and XX cells.

Ribosomal genes of these cells are therefore not only under a control independent from that of euchromatin and heterochromatin but also achieve a fixed final level (relative to the number of doublings of euchromatin) instead of doubling a certain number of times. That the control of ribosomal RNA genes is especially important in the cell has been shown also by the phenomenon of magnification discovered by Ritossa, in which mutants with a reduced number of ribosomal RNA genes seem to be able to increase the number in germ line cells so that the wild type situation is restored. Both lateral extension of the number of genes in one chromosome and the multiplication of the number of chromosome rDNA regions are therefore utilized to maintain a specific content of ribosomal RNA genes in different circumstances.

BACTERIOLOGY

Hospital Bugging

from a Correspondent

HEXACHLOROPHANE has been under attack because it may lead to neurological disorders if used in large amounts for washing babies, and it has been withdrawn from most germicidal soaps. But hospital staff still find it valuable for hand washing and preoperative

Haemoglobin Prediction Verified

THE survey by Perutz of abnormal human haemoglobins has revealed that whenever the mutation involves only an amino acid on the outside of the molecule there are no aberrations in the functional characteristics. An exception to this rule was the β -chain variant, haemoglobin Seattle, which was reported to have glutamic acid in place of alanine at position 76. The side chain in this position is on the surface, and not apparently involved in any intramolecular interactions. Thus either Perutz's scheme of the structure and function relation contained a flaw, or the fingerprinting analysis as published was in error.

The second explanation has now been proved correct. Kurachi *et al.*, writing in *Nature New Biology* next Wednesday (June 27), report the results of a re-examination of the deviant peptide component in the fingerprint map. They find that rather than a glutamic acid for an alanine at position 76, haemoglobin Seattle has aspartic acid for alanine at position 70, which in Perutz's model is in contact with the haem group. The functional disturbance which this substitution induces is not large, the haem

haem instructions and Bohr effect being normal. There is, however, a decrease in oxygen affinity, and an enhanced tendency towards oxidation to methaemoglobin.

In an accompanying letter, Anderson, Perutz and Stamatoyanopoulos describe the structural consequences of the replacement. The difference electron density map reflects the substitution itself, and in addition a displacement of another aspartate side chain and a small disturbance of the haem group, which is tentatively identified in terms of an alteration in the tilt. In the deoxy-form the β carbon and the carboxyl group of the anomalous aspartate side chain are close to two different methyl side chains of the haem. One of these contacts would be lost in the transition of the conformation to that of the oxygenated form.

It is not obvious why these distortions would have the effect that they do on the properties. Anderson *et al.* conjecture that the negative carboxylate group may polarize the haem sufficiently to affect the oxygen affinity, and the increased negative charge could also make it more susceptible to oxidation.

scrubs. It now seems, however, as if hexachlorophane does not give adequate protection against Gram-negative organisms, and thus it can be classified with other cleansers which have been giving hospital workers a false sense of security. For example, Gram-negative organisms have been shown on occasions to be spread in hospitals by cloths and mops—the cleansing solutions being relied on to clean them are inactive against these organisms.

Gram-negative organisms are increasingly becoming a problem in hospital-acquired infections. In the past the most feared organisms were the Gram-positive staphylococci and streptococci which became resistant to the antibiotics in common use. Hexachlorophane has undoubtedly diminished this worry, but is contributing little to the Gram-negative problem.

Two workers at the University of Bergen School of Medicine (Bruun and Solberg, *Br. med. J.*, **2**, 580; 1973) have studied the effect of hexachlorophane and of ordinary bar soaps on the colonization of the hands by Gram-negative organisms. The bacterial hand flora of nursing staff in one medical and two surgical departments were examined for *Staphylococcus aureus* and the Gram-negative organisms *Pseudomonas aeruginosa*, *Proteus* and lactose-fermenting coliforms. Two sets of samples were obtained, with a period of 6 months between them during which one group washed their hands with bar soap only, one with bar soap for 3 months and 3% hexachlorophane for 3 months, and the third with hexachlorophane only. Carriers of Gram-negative organisms were followed with regular sampling.

The results confirm again that hexachlorophane reduces the carriage of *Staph. aureus*. But when all the counts obtained during the hexachlorophane period were compared with counts during the period of using soap only, there was a significantly higher isolation rate for Gram-negative bacilli in the hexachlorophane group. At the start 18.8% of the staff in the survey carried Gram-negative organisms, and it seems that 13% of these continued to carry these organisms for periods of 6 to 20 months. All these persistent carriers were in groups using hexachlorophane.

Unfortunately Bruun and Solberg say nothing about how thoroughly the hands were dried after washing, which is important because Gram-negative organisms flourish best in moist conditions. All the same, the results indicate that hexachlorophane cannot always be relied on. The authors point out that most of the persistent carriers had signs of low-grade infections of the skin of the hands for most of the investigation period, and most of the Gram-negative organisms were isolated from these infected areas.

PLANT GEOGRAPHY

Origin of Cockleburs

from our Plant Ecology Correspondent

THE variation of photoperiodic response found in related plant species from different latitudes is now well documented. One of the most informative genera is *Xanthium* (cocklebur) which has a widespread distribution, but which has an extremely complex taxonomy, chiefly as a result of its morphological plasticity. Ray and Alexander (*Am. J. Bot.*, **53**, 806; 1966) found that *Xanthium strumarium* varied in its photoperiodic response according to the latitude of its origin in North America. Plants of northern origin, for example, Montreal, Quebec, required a critical dark period of only 7.5 h to initiate flowering, whereas plants from the south, for example, Texas, required more than a 10-h dark period. This response is genetically controlled and can be regarded as an adaptation to differing climatic regimes in the North American continent.

McMillan (*ibid.*, **57**, 881; 1970) has since shown variation in photoperiodic response with latitude in Texas, though in Mexico this was not found to be the case. There, considerable variation occurred within a given latitude, which McMillan explains by reference to climatic variability resulting from irregular topography.

Xanthium is a genus of annual plants characteristic of open habitats. It has invaded several areas of the world such as India and Australia as an aggressive weed. Recently McMillan (*Nature*, **240**, 485; 1972) attempted to trace the

geographical origins of the aggressive Indian populations by examining their photoperiodic responses as well as some chemical characteristics. He concluded that the aggressive form was the product of hybridization between indigenous genotypes and a Caribbean taxon, the fruits of which could have been imported in American cotton seed.

McMillan has now turned his attention to the *Xanthium* populations of the Pacific Islands (*Am. J. Bot.*, **60**, 277; 1973), which are regarded as adventives, probably of American origin. He collected seed from plants in Tahiti and Oahu and also from several areas of Mexico at similar latitudes for comparison. Plants grown from the island seeds had a critical dark period requirement of between 10.75 and 11 h. Mexican material showing greatest similarity in response came from the north-western coastal plain in Tamaulipas. Most other Mexican plants had shorter dark period requirements. When grown together at Austin, Texas, in experimental conditions, the populations were shown to differ in a variety of other physiological and morphological characteristics, but the similarity of their photoperiodic requirements gives strong support to the contention that the *Xanthium* populations of the Pacific Islands originated in coastal Mexico. Degener's theory that Hawaiian *Xanthium* was introduced from California now seems most unlikely, because Californian plants have critical dark period requirements varying from 8 h in the north to 9.5 h in the south. The island populations evidently originated in more southerly latitudes.

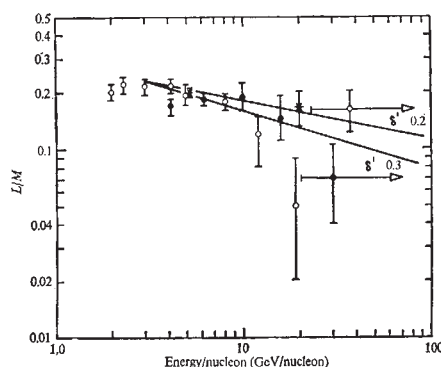
Galactic Escape and Cosmic Electrons

THE implications for cosmic electrons of the discovery that the escape of cosmic rays from the Galaxy is apparently dependent on energy are examined in next Monday's *Nature Physical Science* (June 25) by Silverberg and Ramaty. They come to the conclusion that the effects of the loss of electron energy by the Compton and synchrotron processes on the electron energy spectrum will be

much less than if the "lifetime" in the Galaxy of the electrons is constant at all energies.

The known variation of the ratio of light nuclei ($3 \leq Z \leq 5$) to medium nuclei ($6 \leq Z \leq 8$) with energy is shown in the diagram. The lighter nuclei which are produced by fragmentation of the heavier nuclei during their passage through the Galaxy are less in evidence at higher energies, suggesting that an energy-dependent loss process is at work (or, alternatively, that considerable fragmentation takes place in the sources of cosmic rays).

Assuming that the energy dependence of the loss of electrons from the Galaxy is described by similar parameters to those which describe the effect for nuclei, Silverberg and Ramaty show that the change in electron spectral index resulting from the electron energy loss processes is unlikely to be large at electron energies below several hundred GeV.



GRAVITATIONAL RADIATION

Magnetic Correlation?

from a Correspondent

THE chief evidence for associating the signals recorded by Weber's gravitational wave detectors with an astronomical source outside the solar system has been the 12-hour periodicity displayed in published plots of event rate as a function of sidereal time. Now a wider analysis of the times of some of Weber's events has been made, and this indicates that there may also be correlations with disturbances of the Earth's magnetic field and with sunspot activity. Such correlations would suggest that Weber's detectors may be influenced by phenomena unconnected with gravitational radiation and may help to resolve some of the problems raised by Weber's observations.

The new analysis has been made by Tyson, MacLennan and Lanzerotti of Bell Laboratories (*Phys. Rev. Lett.*, **30**, 1006; 1973), and follows earlier work by Adamyants, Alekseev and Kosonitsyn in the Soviet Union. The Soviet physicists based their analysis on published times of seventeen coincident events recorded by Weber's detectors at Maryland and Chicago in late 1968 and early 1969. They found a correlation between changes of terrestrial magnetic activity and the days when Weber events were recorded and they also found a rather stronger correlation, involving a time lag of 2 days, between sunspot activity and Weber pulses. These results have generally been regarded as interesting, but of limited significance because of the small number of events analysed.

The group at Bell Laboratories was provided by Weber with data on 262 events recorded with his detector in a period of 121 days beginning in August 1969. They have searched for correlations between the daily rates of Weber's events and many other phenomena including geomagnetic activity, sunspot number, averaged earthquake amplitudes at a point between Maryland and Chicago, tidal strain in the Earth and changes in barometric pressure and in temperature. Correlations at levels above 2 standard deviations are found between the Weber data and geomagnetic, sunspot and earthquake activity; with a time lag of 8 days in the sunspot correlation. The strongest geophysical correlation found was with magnetospheric ring-current intensity and was at the level of 2.7 standard deviations. The rate of occurrence of the gravitational wave events throughout the sidereal day was also investigated and a distribution similar to those reported by Weber was obtained, with peaks occurring twice a day at the times when the detectors would be expected to have maximum sensitivity for gravitational radiation

coming from the direction of the galactic centre. This correlation was at the level of 3 standard deviations.

What do these correlations imply? In the case of the magnetospheric correlations the Bell Laboratories group points out that the enhanced low frequency magnetic disturbances which are present at times of high geomagnetic activity may couple into Weber's detectors and produce some "events"; it is known that fluctuations in the magnetic fields can be correlated sufficiently closely in time over distances equal to that from Maryland to Chicago to be recorded as coincident in Weber's equipment. Weber has carried out experiments to search for magnetic coupling to his detectors, but it seems that his published tests were made with magnetic sensing equipment tuned to frequencies related to the resonant frequencies of his detectors. It may be that very low frequency magnetic fluctuations would not be efficiently detected in such tests and might penetrate the steel vacuum tanks and cause vibrations in ferromagnetic materials coupled to the aluminium bars.

It is more difficult to understand the reported sunspot and earthquake correlations, and the Bell Laboratories group does not suggest interpretations here. In fact they do not claim that their analysis indicates the prime source of Weber's events. The point they make is that, as the correlation with some geomagnetic phenomena is almost as strong as the correlation with sidereal time, at least a certain fraction of

Weber's events may be caused by processes other than gravitational radiation.

As the authors indicate, however, the data used in this study represent only a small part of the body of data now collected by Weber and a more extensive analysis might give different results. Clarification of the problem may come from some of the independent experiments with gravitational wave detectors now in progress. None of the results reported to date has confirmed Weber's observations, but most of the experiments have only collected data over relatively short periods, and it may be some time before unambiguous conclusions can be drawn.

MARS

Are Ice Ages Temporary?

by our Cosmology Correspondent

AT a special seminar held at the University of Sussex on June 8, Professor Carl Sagan, of Cornell University, presented some of the evidence from the Mariner 9 photography which has led him to suggest that Mars may be in a temporary ice age at present, and that more clement conditions recur on the planet periodically. This idea has exciting implications for the prospect of finding evidence of life on the planet.

Sagan's discourse was divided into two parts; the first covered the implications of the great dust storm which was raging when Mariner 9 arrived at Mars, and which seems to be a typical pheno-

Spiral Structure and Nuclear Activity in Galaxies

THE density wave theory can explain many features of the spiral structure of galaxies, but requires a regular input of energy. One recent suggestion is that regeneration (about every 10^9 yr) is associated with gas expelled from the galaxy's nucleus. That idea has been followed up by van der Kruit, who describes in *Nature Physical Science* next Monday (June 25) a model in which a continuous cycle links nuclear activity with regeneration of spiral structure, while angular momentum transport by density waves adds to the production of nuclear energy.

Observational data which help in the development of this model have been carried out at Westerbork by van der Kruit, and reveal two important correlations. First, the relative velocity between gas and the spiral pattern, ω_{L0} , is larger when the ratio of radius at which the rotation curve is maximum (R_m) to optical radius (R_0) is smaller. Moreover, a similar relation holds for the compression strength and this ratio; both relations are distance independent.

The Westerbork survey data provide measurements of compression strength

for only fourteen galaxies, but use of the R_m/R_0 relations and of van den Bergh luminosities enables van der Kruit to extend this sample to twenty-six galaxies with well observed rotation curves. Galaxies with low R_m/R_0 , and thus strong compression, are relatively more luminous because of enhanced star formation.

Further observational data suggest that galaxies with low R_m/R_0 also contain better developed nuclei, showing clear signs of activity. Because nuclear explosions can regenerate the density waves by gas expulsion, but these same density waves then transport angular momentum such that gas and rotational energy are fed into the nucleus, van der Kruit suggests that the observations indicate the presence of a closed cycle, both directions of which operate more strongly in galaxies with low R_m/R_0 . There is, of course, some scatter in the observations, and the precise nature of the expulsive mechanism remains unknown. This work points the way to a new and interesting approach to the problem of spiral structure, rather than presenting all the answers.

menon at perihelion. The erosive power of the wind-borne dusts is impressive enough to lend credence to the radar observations from Earth which suggest that large areas of Mars are covered by thick layers of dust and would not form easy landing grounds for vehicles such as Viking. Furthermore, the streaks of light and dark coloured dust which lie in the wind shadows of many of the craters suggest that the dust has a two component structure, as well as providing an insight into the variations of the wind. Thus, it now seems almost certain that the change in shape of the dark splotches on Mars is a result of the movement of light coloured dust over the darker dust; the once favoured idea that the sometimes dramatic growth of these dark areas could be attributed to plant growth now seems untenable.

But if the exobiologists have suffered a disappointment in this regard, Sagan's other news more than made up for it. This centred on the presence of the remarkable sinuous channels which so clearly suggest the presence, at sometimes in the past, of a flowing, low viscosity liquid. The channels are concentrated near the equator suggesting that the liquid in question must be one which can exist, at times, only in that part of the planet. Only one possible liquid fits the bill—water can flow in the equatorial regions of Mars if the planet's temperature is increased slightly. A 15% increase in the heat absorbed at the poles of Mars would produce a self sustaining temperature change, because the release of carbon dioxide would increase the atmospheric pressure, making the climatic circulation which carries warm air from the equator to the poles more efficient, and preventing the CO₂ caps from reforming.

This sort of increase in absorption is by no means impossible. Sagan listed four possibilities:

- Changes in the heat produced by the Sun. This has interesting astrophysical implications which are discussed by Sagan and Young in a letter on page 459 of this issue of *Nature*.
- Changes in the obliquity of the planet's axis to at least 28°. This is consistent with an independent suggestion from planetary astronomers that the obliquity of Mars may vary, up to 34°, with a period of some millions of years.
- Polar wandering, by which the CO₂ ice could be moved into the warmer parts of the planet.
- A decrease in the albedo of the CO₂ ice by only 3%, lasting for at least 100 years.

The last idea is perhaps the most immediately attractive because the albedo of the Martian dust is only about one third of the 75% albedo of the

present polar caps, and there is no doubt that large quantities of dust are frequently stirred up in the Martian atmosphere.

Mars today, then, may be in a temporary ice age. At other times, the planet may be warm enough for running water to flow near the equator, although this would be associated with melting of a permafrost layer rather than rainfall. Obviously, this raises the prospect that some form of life could evolve during the warm spells, and even that spores would survive through the ice ages to await the next warm period. As Sagan pointed out, spores can survive for very long periods on Earth, where there has hardly been the same kind of evolutionary pressure that similar organisms would experience on Mars. Such spores are triggered into activity by the presence of water, and two of the experiments on the Viking landers involve the addition of water to samples brought into the spacecraft. That prospect should certainly rouse still further interest in the Viking missions.

NUCLEAR PHYSICS

Neutrino Interactions

from a Correspondent

THE beauty of the parton model of sub-nuclear matter which was introduced by Feynman is its simplicity. A solid state physicist sees atoms as point-like structures in his solid; as physicists probe further the atoms are seen to have smaller point-like nuclei surrounded by electrons; on closer examination still, nuclei are found to consist of protons and neutrons bound together by the nuclear force. Then the proton and neutron are found to be composite structures themselves with point-like structures within them called partons. In the simplest form of the model these are identified with the three quarks which describe the strong interaction symmetry SU₃.

The predictions of the model are beautifully described in Feynman's lecture note volume *Photon-Hadron Interactions* (Benjamin, New York). The model was constructed to describe the deep inelastic electron scattering measured at the Stanford Linear Accelerator Center (SLAC), which revealed point-like structures possessing spin inside the proton.

New data have now been presented, some of which confirm the model, some which tend to contradict it, and all of which whet the appetite for more information.

At the National Accelerator Laboratory (NAL) at Batavia a neutrino beam has been produced from the 400 GeV proton beam. In a recent *Physical Review Letters* (30, 1084; 1973) the first results are presented of events produced

by neutrinos in spark chambers. Events with a transfer of four momentum to the nucleon of 100 (GeV/c)² are observed, corresponding to distances smaller than 10⁻¹⁵ cm; at this distance the interacting structure still appears to be a point; the number of events caused by antineutrinos is one-third of the number caused by neutrinos, in agreement with low energy data and the simple model. This is stretching the applicability of the model to smaller distances than before, and opens up a new field of physics of which more will be heard—neutrino spectroscopy.

At the Cambridge Electron Accelerator (CEA), experiments with electron-positron colliding beams have been in progress for more than a year. A recent report was given at the Washington meeting of the American Physical Society, and is soon to be published in part in *Physical Review Letters*. As has been already observed at Frascati in Italy, there is a large probability for producing events with many hadrons in the final state. According to the simple parton model, these hadrons are produced with an intermediate state of a parton-antiparton pair. Partons, if they are quarks, have spin quantum number of $\frac{1}{2}$, and the cross-section is therefore related to the cross-section for producing point-like, spin $\frac{1}{2}$, muons, by the relationship $R = \sum q_i^2$; where q_i is the charge of the i th parton. Because the charges on the quarks are $\frac{1}{3}$, $\frac{1}{3}$ and $\frac{2}{3}$, $R = \frac{10}{9}$. Experimentally, Frascati scientists already found $R \approx 1$, but now at CEA it is definitely confirmed that $R = 4.6 \pm 1.1$ at $q^2 = 16$ (GeV/c)², possibly rising to 7 ± 3 at $q^2 = 25$ (GeV/c)².

The parton model can be modified by assuming "sets" of quarks. The first modification allows "coloured" quarks (red, white and blue) and $R = 2$. But this is not enough, and deeper modifications seem necessary which may destroy the model's simplicity—and will certainly change Feynman's book.

The model can also be tested by more precise measurements in a known region. Detailed measurements on the ratio of (e, p) to (e, d) scattering have been made at SLAC, and are also described in *Physical Review Letters* (30, 1087; 1973). These are interpreted as a ratio of (e, p) to (e, n) scattering and the neutron structure can therefore be derived. The neutron and proton, having different charges, are composed of different quarks, so that the experiment is sensitive to these details. A lower bound of 0.25 is imposed on σ_n/σ_p by the parton model. The data show that this is approached at large values of a variable $x = q^2/2MV$, and this is very hard for the simple theory to describe. One should, however, probably only expect the simple theory to describe gross features, and should expect precise experiments to show deviations.

Ageing of Clones of Mammalian Cells

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The chief purpose of this article is to review some recent work on the ageing of mammalian cells and to comment on the theoretical interpretation of the experimental findings. No claim will be made that cellular ageing is a primary cause of the ageing changes observed in whole animals; the evidence supporting such a causal connexion is inconclusive.

HAYFLICK has emphasized a possible connexion between the senescence of clones of mammalian cells maintained in tissue culture, and the ageing of members of the corresponding species¹⁻². He showed that the history of a tissue culture of human fibroblasts can be divided into three phases. His phase I corresponds to the establishment of a primary culture, his phase II to a period when vigorous growth follows each successive subculture, and his phase III to a period in which growth capacity declines until it is impossible to maintain further subcultures. These findings have been confirmed in a number of laboratories.

Hayflick reported that when fibroblast cultures are established from embryonic lung, the cells divide about fifty times before the cultures die out, but that cultures derived from adult lung die after about twenty divisions on average. There was a wide experimental scatter in these experiments, but more recent work has put Hayflick's principal conclusion on a sound statistical basis; the average number of divisions obtained declines by 0.20 for each year of the donor's life³.

Hayflick's experiments and his tentative conclusions have been criticized on a number of grounds. It has been asserted that the limited capacity of human fibroblasts to proliferate in tissue culture reflects deficiencies of the medium rather than any intrinsic property of the cells. Supplementation of the medium, for example, with hydrocortisone^{4,5} or with tyrosine⁶ leads to a substantial increase in the number of doublings that occur before cultures enter phase III. Further, the Hayflick phenomenon is said not to be completely general. Chick fibroblasts, like human fibroblasts, cannot be maintained in culture indefinitely⁷. But it is claimed that a number of diploid cell lines derived from rats have been maintained in culture for well over a hundred doublings, without deterioration or obvious evidence of transformation⁸. This conclusion is disputed¹⁰.

The dependence of the longevity of cell lines on the donor species, the cell type and the culture conditions is important. It warns us not to draw far-reaching conclusions from very limited experimental data. Hayflick's experiments do not show that humans age because their fibroblasts lose the capacity to proliferate after about fifty divisions. Hayflick's own important conclusion, that fibroblasts from adult human donors are reproducibly different from cells from embryos with respect to their proliferative capacity under a wide variety of culture conditions, is, however, not in doubt. More recently it has been claimed that size distribution of human fibroblasts in culture also varies with the age of the donor; phase II cultures derived from adult tissue are said to

resemble phase III cultures derived from embryonic tissue¹¹.

The techniques of tissue transplantation and cell transfer permit the study of the survival and duplication of animal cells over periods longer than the life span of a single animal. The results that have been obtained in this way do not fit any single pattern. In many cases proliferation has been shown to be limited, often to a clonal life span similar to the life span of the animal. In others, tissues have continued to survive in an apparently normal state for much longer than a single life span. The nature of the tissue, the donor species, and the technique of transfer or transplantation all influence the outcome of the experiments¹².

The work of Williamson and Askonas provides a particularly clear-cut example of cellular ageing *in vivo*¹³. They used bone marrow from mice that had been immunized with a DNP-protein conjugate to repopulate acceptor mice whose immune system had been destroyed by heavy irradiation. The technique of focusing electrophoresis was then used to show that, in many cases, the acceptor mice were producing a unique antibody to DNP, presumably because a single clone of DNP-specific cells had become established.

Williamson and Askonas next attempted to carry out serial transfers of bone marrow to one generation after another of irradiated acceptors. They found that the marked clone could be transferred efficiently up to four times. After that antibody production fell off with each successive transfer and the original clone soon died out. It is estimated that the cells of the marked clone went through about ninety doublings in all.

Numerous studies on tissues taken from whole animals have shown that the total activities of many enzymes change with age. These studies, however, have not led to any important generalizations—the activities of some enzymes go down with age, but many enzyme activities are unaffected and some increase^{14,15}.

Studies of the specific activity and thermolability of enzymes, quantities which reflect the quality of the proteins, have given more promising results. When glucose-6-phosphate dehydrogenase (G6PD) or 6-phosphogluconate dehydrogenase (6PGD) from young cultures of MRC-5 fibroblasts is heated, enzyme activity decays exponentially, showing that the corresponding enzyme is homogeneous. The activities of these enzymes when isolated from cells in phase III, on the contrary, decay in a way that reveals the presence of up to 25% of abnormally thermolabile protein¹⁶. In related experiments it has been shown that the specific activity of lactic dehydrogenase isolated from cultures of MRC-5 cells in phase III is lower than that of the same enzyme isolated from cultures in phase II¹⁷. Gershon and Gershon have found similar alterations with age in the properties of the aldolase isolated directly from rat liver¹⁸ and of isocitrate lyase from a nematode¹⁹. They interpret their results to show that the materials obtained from old animals consist of mixtures of an unaltered protein and a unique altered species.

In their experiments on human fibroblast cultures Holliday and Tarrant found¹⁶ (1) that the proportion of thermolabile protein increases with the age of a culture (Fig. 1); (2) that the ratio of the proportion of thermolabile G6PD to the proportion of thermolabile 6PGD remains constant as cultures age; (3) that treatments which arrest the growth of cells, for example deprivation of serum, do not cause the

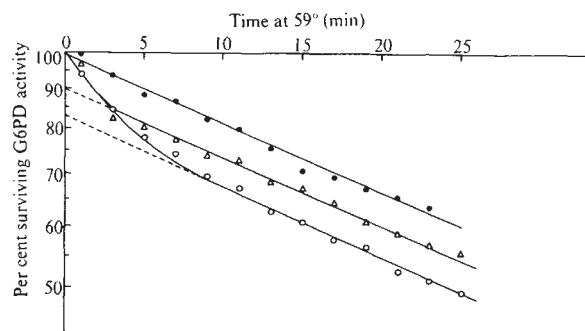


Fig. 1 Inactivation at 59° C of glucose-6-phosphate dehydrogenase obtained from young, middle aged and senescent cells. ●, Passage 22; △, passage 48; ○, passage 61. Reproduced with permission from Holliday and Tarrant¹⁶.

accumulation of thermolabile protein; (4) that cells grown in the presence of low levels of 5-fluorouracil, a base analogue known to induce errors of protein synthesis, age prematurely. The ageing cells accumulate thermolabile protein after a relatively few subcultures; (5) that tissue cultures derived from a donor with Werner's syndrome already contain substantial amounts of thermolabile protein when they enter phase III after less than ten subcultures (R. Holliday, personal communication).

Holliday's results taken together with those of Lewis and Tarrant make it very probable that MRC-5 cells in phase III contain substantial amounts of protein that differ from the standard proteins present in cells in phase II. It seems unlikely that thermolabile isozymes of two independent enzymes are produced in old cultures, so aberrant protein is presumably present. These experiments, however, do not establish that protein synthesis is inaccurate. An alternative explanation is that post-synthetically modified proteins accumulate in old cells.

It is theoretically possible that the thermolabile proteins in old cells are derived entirely, or in part, from non-dividing (dead) cells and represent a protein fraction that is partially denatured or partially degraded. The enzymatic deamination of asparagine and glutamine residues, if it occurred at random positions in proteins, could well produce the observed results. A mechanism of this kind is not excluded by control (3) because serum-deprived cells, unlike old cells, might not contain the relevant degradative enzymes in an activated form. The experiment with 5-FU is not conclusive because the induction of thermolabile protein by 5-FU might be an indirect consequence of cell death rather than a direct consequence of errors of protein synthesis induced by the analogue. Recent experiments by Holliday (unpublished) argue against this explanation. They suggest that the proportion of thermolabile protein in moribund cultures is less than in cultures in phase III.

If it turns out that, in old cultures, newly synthesized proteins do have incorrect sequences, it will then be necessary to decide whether inaccurate protein synthesis is responsible, or whether the cultures have accumulated a wide variety of independent mutations in the G6PD and 6PGD genes. Holliday's results on the rate of appearance of thermolabile enzymes are not easily explained in terms of somatic mutations, but they do not completely exclude such an explanation. The distinction between mutations and errors of protein synthesis could be made by studying clones derived from single cells, by studying the components of the protein synthetic apparatus *in vitro*, or by examining virus-specified proteins.

It seems likely, on theoretical grounds, that mutations accumulate in tissue cultures as they age. The evidence on this point, however, is restricted to preliminary observations

by Fulder²⁰, who has shown that glucose-6-phosphate dehydrogenase mutants are more common in old than in young human fibroblast cultures. There is a good deal of evidence that the frequency of gross abnormalities in chromosome structure increases with age in cells taken from whole animals²¹.

Old tissues differ from young ones in another way that implies a difference in their macromolecular composition. There are many indications that the induction of enzymes in old tissues is abnormal. The induction of tyrosine aminotransferase in old tissues, for example, is slower than in young, although the same rate of synthesis is attained eventually²². It is not possible, at present, to decide whether such differences in the rates of induction originate in changed nucleic acids, in changed quantities of the various proteins that control gene expression, or in a change in the accuracy with which control proteins are synthesized.

Theoretical Considerations

(a) Organelles. The various theories of clonal ageing are usually supposed to explain modifications of nuclear DNA or cellular protein that occur in cultures as they age. Such theories are equally applicable, however, to changes in the corresponding components of the mitochondria, because mitochondrial DNA and some mitochondrial proteins are synthesized by more or less independent mitochondrial systems. It is possible that mitochondria are more susceptible to the effect of mutation or errors of protein synthesis than are the cells in which they are located. If so, the ageing of the cells might be a consequence of the deterioration of their mitochondria. In the blowfly, ageing is accompanied by the uncoupling of oxidative phosphorylation, but the nature of the molecular changes that occur in the mitochondria has not been determined²³.

(b) Subcultures and cell divisions. The terminal stage in the senescence of a tissue culture is usually identified as the point at which it becomes impossible to split a sample of cells into two equal portions and to regenerate the original number of cells from one of the halves. But each sub-clone does not go through a number of divisions equal to the number of subcultures, for a proportion of non-dividing cells is always present in fibroblast cultures. The relatively small scatter in the number of subcultures obtained before death is, in part, a consequence of an averaging procedure that is implicit in the design of the experiment. As the cultures get older, the proportion of healthy cells decreases, so those cells that can still divide must go through an increasing number of divisions, on average, before the total cell number doubles. This, so to speak, compresses the period of senescence into a few subcultures, although the scatter in the number of cell divisions that can occur before the death of a sub-clone may be much larger. A similar compression may occur *in vivo*.

The heterogeneity of the cell populations must be borne in mind when interpreting the results of biochemical experiments on ageing cultures. The differences being average properties of young and old cultures may give more information about cells that have ceased to divide than about the cells which are responsible for continued growth. It is important to distinguish progressive changes that accompany the ageing of mitotically competent cells from catastrophic changes that follow cell death (release of lysosomal enzymes, and so on).

(c) Dilution theories²³. The simplest of all the mechanisms that have been proposed to explain clonal ageing, the dilution of an essential and irreplaceable component of the primary cells, is ruled out on numerical grounds—the molecules would be diluted out long before the fiftieth division. A closely related mechanism, the dilution of an essential component that is synthesized at a rate that just fails to keep pace with cell division (the mitochondria, for example), is compatible with what we know about ageing, but does

not seem very plausible. The simple accumulation of damage also fails to account for the ageing of dividing cells, for damaged molecules would be diluted out by newly synthesized material. This last argument does not hold for non-dividing cells, and it is possible that the ageing of neurones, for example, is due to postsynthetic damage to DNA, proteins, or other cellular components.

(d) Molecular clocks and programmed ageing. It has been suggested that the ageing of the individual, far from representing an inevitable consequence of the limited accuracy of biological processes, has a positive adaptive value for the species. Thus, well-defined mechanisms may have evolved that guarantee senescence at an appropriate time. These ideas lead naturally to the notion of a molecular clock, a device that counts cell divisions (or elapsed time) and triggers an irreversible ageing process when the optimal life span is approached²⁵.

Very little is known for certain about the factors determining reproductive life span and longevity in different species. It is even uncertain that ageing is a significant phenomenon in wild populations; it is said that few animals survive long enough to be able to age. It is not clear that senescence has a positive adaptive value, so it seems unwise, in the absence of experimental evidence, to take for granted the idea of "programmed obsolescence" in man and other mammals²⁶.

The one great merit of the molecular-clock theory is that it accounts readily for the phenomenon of transformation. If cells are programmed to age, a mutation that interfered with the programme could make them "immortal". Similarly, a transforming virus might interfere with the natural ageing programme. We shall see that the phenomenon of transformation can be explained only with some difficulty on the basis of error theories.

(e) Elementary protein error theory. There are two steps in protein synthesis involving discrimination between related molecules. Each activating enzyme must select a unique amino acid and load it onto an appropriate tRNA. A codon of messenger RNA must pair with the anticodon of an appropriate tRNA, but not with any inappropriate anticodon. Each of these processes is associated with a small but non-zero error frequency. Errors in RNA synthesis could also decrease the fidelity of protein synthesis.

Because the proteins that are part of the protein-synthetic apparatus are synthesized in the same way as other cellular proteins, errors in protein synthesis could be self-propagating²⁷. Suppose we started with an error-free first-generation protein synthetic apparatus and used it to synthesize activating enzymes, ribosomal proteins, and so on. The second generation protein synthetic apparatus would certainly contain some errors. If we used this error-containing second generation protein synthetic apparatus to synthesize more activating enzymes, and so on, they would contain even more errors.

If the cycle discussed above was repeated many times, each successive generation of protein synthetic apparatus being used to synthesize the components of the next generation, one of two things would happen. Either the error-frequency would converge to a stable non-zero value, or it would diverge. In the former case, no ageing phenomenon would occur, but in the latter the error frequency would, sooner or later, become so large that the cell could no longer function.

When I first discussed this problem I thought that in the absence of cellular selection, the error frequency of protein synthesis would certainly diverge. I no longer believe this to be the case. It is possible that a protein synthetic apparatus containing a relatively small number of errors might be able to synthesize a new protein-synthetic apparatus containing fewer errors. This must have been true at some stage in the evolution of the genetic apparatus, unless cellular selection became important very early. The exist-

ence of enzymes that scavenge error-containing proteins makes it more plausible that a small, stable error frequency can be maintained²⁸.

In only one case is there direct experimental evidence that the frequency of errors of protein synthesis increases rapidly and leads to cell death. The *leu-5* mutant of *Neurospora* synthesizes a temperature-sensitive leucine-activating enzyme which, at higher temperatures, allows other amino acids to substitute for leucine²⁹. At low temperatures protein synthesis proceeds more or less normally. The *leu-5* strain, unlike the wild-type, senesces when maintained at 35° C.

Lewis and Holliday have shown that the accuracy of protein synthesis in *leu-5* falls slightly when this mutant is shifted from 25° C to 35° C, and then remains almost constant for a considerable time. After about 70 h the cells age rapidly, and at the same time the thermolability of glutamic dehydrogenase increases, while its specific activity falls dramatically. The "error-catastrophe" hypothesis fits all of Lewis and Holliday's extensive observations on this system³⁰.

There is, as yet, no experimental evidence concerning the mechanism of senescence in fungi that age spontaneously in nature, for example, *Podospora*. We have already seen that the origin of thermolabile protein in ageing fibroblast cultures is unclear. If an error catastrophe is the cause of ageing in non-mutant fungi or in mammalian cells in culture, this can only be established by the measurement of the fidelity of protein synthesis.

(f) Error theory—more general models³¹. Holliday and Tarrant have remarked¹⁶ that it may not be possible to separate the contributions to cellular ageing caused by errors of protein synthesis from those due to the accumulation of somatic mutations. Errors of protein synthesis must occasionally lead to the formation of "mutator" DNA polymerase molecules which replicate DNA inaccurately. Conversely, some mutations, for example, the *ram* mutations in *E. coli*³², must reduce the fidelity of protein synthesis. Thus inaccurate protein synthesis and inaccurate DNA synthesis are coupled phenomena.

If the coupling between different causes of ageing is sufficiently strong, we cannot use a conventional description in which the deterioration of one process, say, protein synthesis, is thought of as primary and the cause of the subsequent breakdown of other processes such as DNA replication. Instead we must use a more general description in which the fidelity of each of a set of significant processes is dependent on the fidelity of some or all of the others.

A theory of this kind forms a natural generalization of the simple protein-error theory. It retains the central idea of positive feedback—the greater the number of errors that have accumulated in the macromolecular constituents of the cell, the faster the accumulation of further errors. At the same time it permits one to consider the effect of mutations, relaxed control processes, faulty membranes, and so on, in parallel with errors of protein synthesis, without assigning priority to any one process.

The coupling between different contributions to ageing must also be important in the senescence of whole animals. The deterioration of extracellular components (collagen, say) is likely to change the intracellular environment in such a way as to reduce the fidelity of macromolecular synthesis. The inaccurate synthesis of proteins would surely have important effects on the condition of extracellular components. It follows that extracellular and intracellular mechanisms of ageing are coupled. I believe that the theoretical and experimental analysis of strongly coupled deteriorative processes will do much to clarify our ideas on the nature of mammalian ageing.

(g) Advanced mathematical methods. If errors of macromolecular synthesis are established by experiment to contribute to ageing, a more sophisticated mathematical treatment of the subject will be justified. The overall error fre-

quencies and mutation rates which we have discussed will need to be replaced by error matrices, which specify the probability that any given amino acid A_1 (or nucleotide, N_1) is replaced erroneously by a different amino acid, A_2 (nucleotide N_2). It is also likely that simple treatments which utilize difference or differential equation will be replaced by a more general stochastic theory.

(h) Transformation. Mammalian cells in tissue culture are transformed by certain viruses, by chemical carcinogens, or spontaneously to permanent or transformed lines which can be subcultured indefinitely. It seems unlikely that DNA replication or protein synthesis is made more accurate by each of these modes of transformation. Thus, at first sight, the phenomenon of transformation seems incompatible with error theories of clonal ageing.

In fact, the situation is more complex. The switch from a "spanned" culture to one that is "immortal" does not necessarily signify a qualitative change in other cellular properties. An elementary mathematical result from the theory of branching-chain processes shows that if cells continue to divide and to produce, on average, more than one viable descendant at division, then cell cultures are potentially immortal. On the other hand, if each cell sooner or later comes to produce on average less than one mitotically competent descendant, the cultures inevitably die out. Thus a "spanned" culture would be made "immortal" by any change that increased the mean number of viable descendants sufficiently. It is important to note that this result is completely independent of the mechanism which leads to the increase in the number of viable descendants.

Consider first a very artificial situation, a culture of *E. coli* growing while exposed to a source of high-energy radiation. Suppose that each cell accumulates a lethal mutation in the time $t_{1/2}$ with probability 0.5. If cells are maintained under conditions which permit doubling in a time less than $t_{1/2}$, cultures are "immortal". Otherwise, they are "spanned". A small change in the medium could, therefore, "transform" *E. coli* in these rather special conditions.

This result cannot be applied directly to error theories of ageing, but it does suggest the sort of explanation of transformation that is needed. It must be postulated that an increased mitotic rate or the escape from contact-inhibition provides a protection against the accumulation of errors because, as in the simple example, it permits more cellular selection. This would be true if, for example, the rate of accumulation of errors (in proteins or nucleic acids) includes a term that is linear in the time (for example, a term describing errors occurring in non-dividing cells) while selection is entirely of a cellular type and occurs only through cell division.

A theory of this kind would be complicated and its predictions would depend crucially on the details of the postulated model of error accumulation, for example, on the ratio of the rates of error accumulation in stationary and non-stationary phases. It is almost certain that a stochastic treatment would be needed to obtain significant results. Some predictions, however, seem general. Transformed cultures, in spite of their so-called immortality, should generate slowly dividing or non-dividing cells. If errors of protein synthesis are relevant, transformed lines should "revert" in the presence of amino acid analogues.

If errors accumulate in somatic cells and finally lead to the cessation of cell division, why does the same process fail to occur in the germ line? The simplest escape from this dilemma is to suppose that protein synthesis is sufficiently accurate in germ-line cells to maintain a constant, low error level. In somatic cells the rate may be higher, or may become higher with age, so that an error catastrophe ultimately develops.

It is also possible that "quality control" processes operate during oogenesis and early development, and lead to the

rejection of ova or embryos in which the error level is too high. It is known that, in humans, many of the oocytes that begin to mature later degenerate. This could be the result of cellular selection for "metabolic efficiency".

Future Developments

Techniques are now available that permit the measurement of mutation rates and error frequencies in fibroblast and other cell cultures. We can expect to know fairly soon whether or not these quantities vary with the age of the cultures in the manner predicted by error theories. It is important that information of this kind be accumulated for as wide a range as possible of cell types and animal species.

Such information, while valuable, will not be sufficient to enable us to decide whether or not errors of molecular synthesis are among the primary causes of clonal ageing, or whether they are triggered by some different molecular process. Studies of the division times within individual clones of cells may help to resolve this issue. Protein-error theory, for example, would lead one to expect a positive correlation between the division times of mother and daughter cells, even in young or transformed cultures—molecular clock theories do not require such a correlation.

It is still difficult to measure the fidelity of macromolecular synthesis in the tissues of whole animals, but information of this kind should become available soon. Even when we have this information, the difficulty of determining the relevance of such experiments to ageing will be formidable. A low level of errors in any particular type of cell would not rule out the possibility that ageing is due to error accumulation in some other type of cell. Conversely, if the error level is high in some or all tissues of old animals, this could be the consequence of some earlier triggering event, and might have little or no importance for the pathology of ageing.

A connexion between the ageing of cells and the ageing of animals would be suggested if it could be shown that a process that increases the life span of cells in culture produces a corresponding increase in the longevity of the donor species. The demonstration that genetic diseases characterized by premature ageing are associated with inaccurate DNA, RNA or protein synthesis would also count as evidence in favour of a contribution of errors of synthesis to whole-animal ageing.

I believe that it will be a considerable time before the importance of intracellular errors of macromolecular synthesis for *in vivo* ageing can be assessed. It seems clear that immunological mechanisms and the deterioration of extracellular material play an important part in the ageing of mammals. It may be difficult to determine whether these processes are independent of errors of macromolecular synthesis, coupled to them, or a consequence of them.

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Muscular Contraction and Cell Motility

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It has become apparent during the past few years that close similarities exist in a number of instances between some of the proteins directly involved in contraction in striated muscle, and proteins present in certain non-muscle cells in which movement occurs. The question therefore arises whether all these systems share a common basic mechanism, and if so, whether the current picture of the contracting mechanism in striated muscle, which is a fairly detailed one in some respects, can cast any light on these other motile mechanisms, which are less well understood.

Here I first describe certain aspects of the muscle mechanism, especially structural ones, which seem to me particularly relevant to more general questions of motility. Next, I review a number of recent studies which demonstrate in a very decisive way that the similarities between the proteins concerned are ones that relate to the most basic properties and interactions used in the muscle mechanism. Finally, I point out that these considerations suggest a definite mechanism for certain kinds of cell motility. This general type of mechanism ("active shearing") has been suggested by others before¹⁻³ (though not always in very explicit terms) but it does not seem to have gained general acceptance.

According to the sliding filament model of muscle contraction striated muscles consist of overlapping arrays of actin and myosin filaments which can slide past each other when the muscle changes length. The individual filaments, and the arrays which they form because of their in-register arrangement, remain virtually constant in length. The active sliding force between the filaments is developed by cross-bridges on the thick myosin-containing filaments. These represent the biologically active ends of individual myosin molecules, which can attach to, and exert a longitudinal force on, the thin actin-containing filaments alongside. A cross-bridge is believed to act in a cyclical manner, pulling an actin filament along towards the centre of an A-band for a distance which is probably of the order of 50 to 100 Å, then releasing and reattaching to the actin filament at another point, initially further away from the centre of the A-band and going through the cycle again.

A continuous movement of the actin filament is thus produced by the asynchronous action of all the cross-bridges acting upon it from the myosin filaments alongside.

The mechanism requires that the molecules of myosin and actin are assembled in their respective filaments with the appropriate structural polarity. As the cross-bridges have to move so as to draw the actin filaments towards the centre of the A-band, they must be oriented so that they pull in one direction in one half A-band and in the opposite direction in the other half. Thus all the myosin molecules must be oriented in one sense in one half of the length of each thick filament, and in the opposite sense in the other half. This is indeed found to be the case in practice⁴. Also myosin molecules are able to assemble *in vitro* into filaments with this important and characteristic reversal of polarity half way along their length. A similar requirement applies to the actin filaments. Highly specific interactions between an actin monomer and a myosin cross-bridge require that the interacting groups on the two molecules always have the appropriate mutual orientation. Thus actin filaments which interact with opposite ends of a myosin filament must contain actin monomers with opposite polarity. In practice, it is found that all the actin monomers along a given actin filament have the same polarity, and that the polarity reverses at the Z-lines. Thus the actin filaments are attached on either side of the Z-lines with opposite polarity⁴.

This means that the direction of the relative force experienced by an actin filament when it interacts with appropriately oriented myosin filaments is specified by the structural polarity of the actin filament itself. Consequently, if an actin filament were attached to some other cellular structure with the same polarity as actin filaments are attached to Z-lines, then the attachment point would experience a force pulling it in the direction of the actin filament. Again, as the force exerted on an actin filament must always be in the same direction, such a filament (or group of similarly polarized filaments) might be maintained in motion over significant distances by interaction with myosin filaments⁴, whose orientation could be selected and perhaps enforced by the actin. These considerations are obviously relevant to mechanisms for cell motility and will be discussed again later.

A second characteristic of the sliding filament mechanism as it appears to work in practice is that the relative force between myosin and actin is developed as the result either of an active change in the angle of attachment of the head of the myosin molecules (the S₁ subunit) to the actin filament (Fig. 1), or of an active change in the shape of the S₁

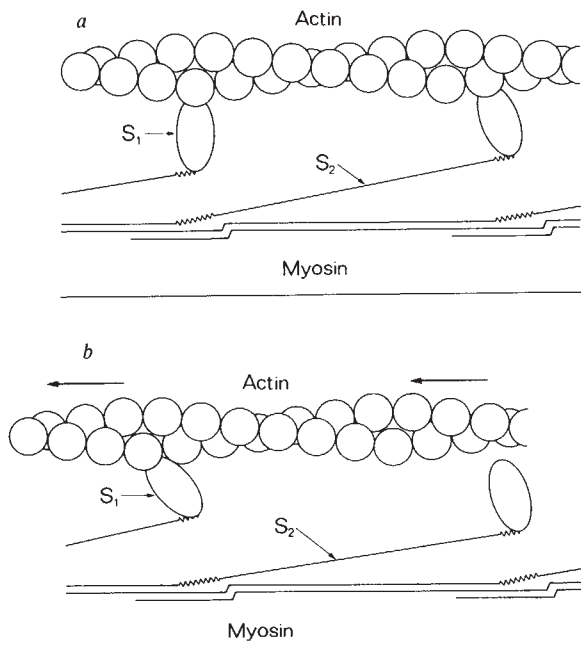


Fig. 1 Active change in angle of attachment of cross-bridges (S₁ subunits) to actin filaments could produce relative sliding movement between filaments maintained at constant lateral separation (for small changes in muscle length) by long range force balance. Bridges can act asynchronously as subunit and helical periodicities differ in the actin and myosin filaments. Only one of the two S₁ subunits in each myosin molecule is shown. *a*, Left hand bridge has just attached; other bridge is already partly tilted. *b*, Left hand bridge has just come to the end of its working stroke; other bridge has already detached, and will probably not be able to attach to this actin filament again until further sliding brings helically arranged sites on actin into favourable orientation.

subunit, the attachment to actin remaining rigidly fixed⁵. That is, the relative sliding force appears not to be generated by changes elsewhere in the myosin molecule or filament, not, for example, by an active change in the orientation of the S₁ head generated at the link to the S₂ part of the molecule. The arguments supporting this view, which I believe are very powerful are somewhat involved, and the original papers should be consulted^{4,6,7}. In essence, they derive mainly from X-ray diffraction and electron microscope evidence that the attachment of the heads of the myosin molecules to the backbone of the thick filament is very flexible, whereas their attachment to the actin filaments (in the rigor configuration) is a very rigid one.

If this picture is correct, it follows that the basic contractile mechanism is represented by the interacting complex of thin filament and attached myosin head, and that the exact mode of assembly of myosin molecules into filaments, provided their appropriate polarity is maintained, may be capable of some variation. Even amongst striated muscles, variations are observed in non-vertebrate species, and it is perfectly possible that myosin may be organized in quite different forms in other situations whilst the same myosin head-actin filament interaction is still maintained.

A third feature of the sliding filament mechanism in striated muscle is concerned with the exact mode of attachment of the myosin head to the actin filaments. At present, no means are available to arrest the working stroke of the cross-bridge, or to prevent subsequent dissociation in the presence of ATP. In the absence of ATP, however, the myosin cross-bridges remain attached to the actin filament and the muscle is then in rigor. In an *in vitro* system, actin filaments can be "decorated" in the absence of ATP, by the attachment of molecules of myosin or of heavy meromyosin (HMM) or of subfragment 1 (S₁). Suggestion of an "angled" configuration of the attached

myosin heads was given by the visual appearance of "arrow-heads" on decorated thin filaments, but the first good evidence was provided by electron microscope studies of thin sections of glycerinated insect flight muscle, and of the X-ray diagrams given by it in rigor and in the relaxed state⁸. More detailed studies of the negatively stained decorated filaments by the three-dimensional image reconstruction technique⁹ have shown that not only are the myosin S₁ subunits (which appear as somewhat elongated structure of dimensions very approximately 150 Å × 40 Å × 30 Å) tilted at an angle of about 45° to the long axis of the actin filaments, but they are slewed round in a characteristic way, that is rotated by about 45° in a plane parallel to the axis of the actin filament and perpendicular to the plane in which they are tilting. It is this combination of tilting and slewing, together with the distinctive shape of the myosin heads, which gives rise to the very characteristic "arrowhead" appearance (Fig. 2), which would not arise from a straightforward "tilted" attachment. We do not at present know the functional significance of this peculiar feature of the attachment (the tilt itself is, of course, what we should expect to find at the end of the working stroke if the initial attachment was perpendicular, and is in the right direction); but it is diagnostic of a very specific structural interaction and of a very specific shape of the attached molecule. It has been found in many cases, as I will describe, that proteins which may be involved in non-muscular motility can form complexes of virtually identical structure.

A final feature of the contraction mechanism in striated muscle is concerned with regulation of activity, that is with switching contractile activity on and off. This is effected by preventing the attachment of the myosin heads to actin in a relaxed muscle, and allowing it to take place when the muscle is activated. Attachment to actin is a necessary part of the biochemical cycle in which ATP is split and force developed by the actin-myosin system. In its absence, ATP splitting, by the myosin alone, takes place very slowly and no tension is developed by the sliding filament system. Indeed, the filaments will slide past each other passively under the action of relatively small external forces.

In a relaxed muscle, the concentration of free calcium is kept at a very low value (probably less than 10⁻⁷ to 10⁻⁸ M) by the action of the calcium pump in the sarcoplasmic reticulum. Upon activation, calcium is released so that the level of free calcium rises (probably to 10⁻⁶ to 10⁻⁵ M) and attachment of myosin to actin can take place. In vertebrate striated muscle, this change is effected by the troponin-tropomyosin system in the actin-containing filaments¹⁰, which prevents attachment of myosin in the absence of free calcium, but allows it to take place in its presence, possibly by a steric blocking mechanism dependent on changes in position of tropomyosin. In molluscan muscles, however (including the striated muscle of *Pecten*), regulation is effected by changes in the myosin molecules to which calcium ions become bound upon activation¹¹. The distribution of these two types of regulation, or combinations of them, between species is a subject of very active current research¹². Nevertheless, all systems so far investigated share the common feature that they are operated by changes in the concentration of free calcium ions over the critical range of 10⁻⁷ to 10⁻⁵ M.

If one is primarily interested in the molecular mechanism of contraction, then striated muscle is the best system to work with, because of its high degree of order, because of the high concentration and relatively large amounts of the contractile proteins in it, and because of its great robustness and stability, which allow it to be manipulated with ease and fixed for electron microscope examination in a state close to its native one by relatively crude techniques. We must now consider, however, whether the same basic mechanism appears anywhere else in nature,

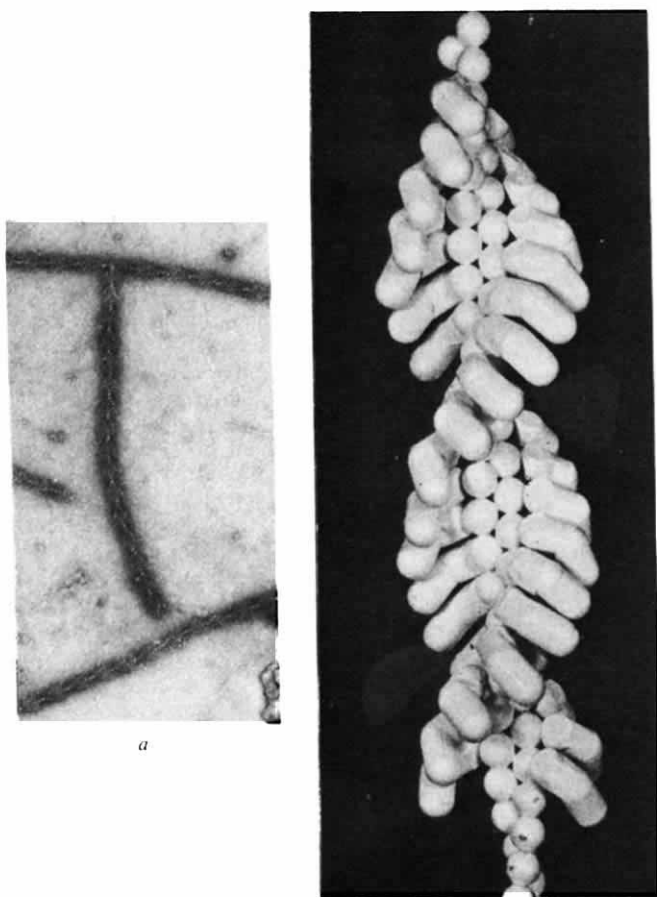


Fig. 2 *a*, Electron micrograph of negatively stained preparation of actin filaments "decorated" with myosin subfragment 1, showing well developed "arrowhead" formations ($\times 138,600$). *b*, Simplified model of "decorated" actin filament, based on 3-D reconstruction results; the S_1 subunits are attached to the central core of helically arranged actin subunits in a characteristically tilted and slewed configuration, and it is this that leads to the appearance of arrowheads.

even, in the first instance, in as closely related a system as smooth muscle.

There is no difficulty here with the kind of smooth muscles found, for example, in the adductor and retractors of bivalves; these have been shown to contain abundant thick and thin filaments¹³, and the reason that striations are absent is the simple one, suggested by Jean Hanson and myself¹⁴, that the filaments are not arranged in register. It is vertebrate smooth muscle which has until recently presented much more difficulty, as for many years only thin filaments could be seen by electron microscopy and only actin reflexions picked up by X-ray diffraction, in spite of the fact that both actin and myosin were obviously present from biochemical observations.

In the past few years, however, several groups of workers have shown that the form of the myosin component in vertebrate smooth muscles is very dependent on the physical and chemical environment of the muscle; and Lowy *et al.*^{15,16} have found that with appropriate conditions before and during fixation, prominent and plentiful ribbon shaped structures can be seen amongst the actin filaments. They have also shown that there is good X-ray evidence for this form of myosin aggregate in comparable specimens before fixation. Some workers believe that cylindrical filaments represent the more natural form of the myosin assembly in smooth muscle, but I think everyone now agrees that myosin is present in smooth muscle in the form of large aggregates and that this probably represents its form when involved in contraction.

The actin component and the regulatory protein system appear to be virtually the same as in vertebrate striated muscle, and the main difference detectable in the purified proteins from smooth muscle lies in the solubility properties of the myosin, that is, its inability to form aggregates with itself at physiological ionic strength except in special conditions. We do not know the functional significance of this, but it is undoubtedly responsible for the difficulties which have occurred in detecting the myosin component by X-ray diffraction and electron microscopy. However, I do not think that there are any serious doubts now that contraction takes place in smooth muscle by the same basic molecular mechanism as in striated muscle and by an essentially similar sliding filament process, although the overall structural organization may be somewhat different.

Non-muscular Systems

Before I consider how some of the other motile systems might function mechanically, it will be helpful to review briefly some of the more recent findings on their protein components. This account cannot do justice to many of the pioneers, but very often technical advances have now made it possible to characterize these proteins much more fully and relate them to the proteins from muscle in a decisive way; and this latter evidence is easier to summarize in a short article.

First, consider the amoeba *Acanthamoeba castellanii*, which has the capacity for typical amoeboid movement. Weihing and Korn¹⁷⁻¹⁹ have extracted an actin-like protein from this organism, which is virtually identical to muscle actin in every respect that can be investigated. It forms the same double helical filaments when examined by negative staining in the electron microscope, and these give exactly the same arrowhead structures when combined with muscle myosin, showing that the myosin-binding sites are oriented in a closely similar way²⁰. The amoeba actin can activate the ATPase activity of muscle myosin, possibly to a lesser extent than muscle actin can, and this can be regulated by the tropomyosin-troponin system of rabbit muscle²¹. When the molecule is cleaved by cyanogen bromide, three large peptides can be isolated which are almost identical in amino acid composition to the three corresponding cyanogen bromide peptides from muscle actin¹⁹. As in muscle actin, one of these peptides contains the rare amino acid 3-methylhistidine. Additionally, amoeba actin contains one residue of ϵ -N-dimethyllysine and small amounts of ϵ -N-monomethyllysine.

Next consider the slime mould *Physarum polycephalum*, which shows very active cytoplasmic streaming. It has been shown by Hatano *et al.*²²⁻²⁴ that an actin-like protein can be extracted from this organism, which shows the same double helical structure as muscle actin in the electron microscope. As in muscle actin, the monomeric form has one mol of bound ATP which is dephosphorylated to ADP when the actin polymerizes in the presence of salts. Adelman and Taylor²⁵ have shown that slime mould actin can activate the ATPase activity of muscle myosin, and Nachmias *et al.*²⁶ have shown that the actin can combine with rabbit muscle myosin subfragment 1 to give arrowheads very closely similar to those characteristic of muscle actin. Again, this shows a very specific structural relationship in the complex.

Next consider blood platelets, which undergo a kind of contraction during clot retraction. Several groups of workers²⁷⁻³⁰ have shown that the protein known as thrombosthenin A is closely similar to muscle actin in its structure, in its ability to form arrowhead complexes with muscle myosin (usually HMM), in its ability to activate muscle myosin ATPase, in its ability to allow its activating activity to be regulated by the tropomyosin-troponin system, and in its content of 3-methylhistidine.

Again, consider nerve cells actively growing and extending in tissue culture. Fine and Bray³¹ have shown that no less than 20% of all the "soluble" protein in developing chick neurones behaves like actin on an SDS gel. This protein forms the characteristic arrowheads with HMM and shows remarkable chemical similarity to muscle actin on two-dimensional electrophoresis diagrams; of fourteen methionine labelled peptides, ten coincided with ones from muscle actin, showing close homology though not necessarily complete identity.

Further examples could be given, but I think the cases mentioned are sufficient to show decisively that in a number of widely different cells exhibiting different forms of motile activity, a protein closely similar to muscle actin by rather strict criteria is present in significant amounts. And in many cases, the protein can be identified in the cell *in situ*, as bundles of filaments of the appropriate diameter, which can be "decorated" by HMM, and which are in a position where they might be associated with movement³²⁻³⁴.

In addition to non-muscle actins, myosin-like proteins have also been found in many of these same cells possessing motile activity, and in several instances the protein in question has been identified by rather strict criteria.

In the slime mould *Physarum polycephalum*, Hatano *et al.*³⁵⁻³⁷ have isolated a protein which has similar ATPase and actin-combining activities to muscle myosin, the same very characteristic form (being a long rod-shaped molecule with a globular region at one end), and a similar sedimentation constant to muscle myosin (~6.0S). Interestingly, the protein differs from striated muscle myosin in being soluble at physiological ionic strength. Adelman and Taylor²⁵ showed that its molecular weight was close to that of muscle myosin, about 460,000. Nachmias^{38,39} showed that the slime mould myosin would attach in the characteristic arrowhead configuration both to slime mould actin and to muscle actin, and also⁴⁰ that in the presence of millimolar concentrations of calcium the myosin would assemble itself into rather short but essentially similar bipolar filamentous aggregates like those of muscle myosin.

In the case of blood platelets, Bettex-Gallard *et al.*⁴¹ and Booyse *et al.*⁴² have shown that the other component of thrombosthenin is a myosin-like protein, having calcium and magnesium-activated ATPase activity and a molecular weight of approximately 540,000. Adelstein *et al.*²⁸ have shown that this molecule contains large polypeptide chains of chain weight 200,000, just like muscle myosin, and chains of 16 to 18,000 molecular weight, analogous to the light chains of muscle myosin. The platelet myosin will form excellent arrowhead structures with actin, and will also assemble itself into the characteristic bipolar aggregates at low ionic strength.

In the case of the amoeba *Acanthamoeba*, however, Pollard and Korn⁴³ have found that, although a myosin-like component can be identified in the sense of having ATPase activity and actin-binding ability, this protein is rather different from muscle myosin in other respects, having a much lower molecular weight (~200,000) and lacking the ability to form filaments. This particular species of amoeba may, of course, be a special case, and it should be remembered that myosin-like filaments have been seen in other amoeba (for example, in *Chaos chaos*^{44,45} and in *Proteus*^{46,47}).

Thus there are a number of instances where it has been demonstrated quite decisively that cells showing various forms of motility contain proteins which behave in many crucial respects exactly like actin and myosin from muscle. The resemblance is particularly significant in those properties concerned with the activating effect of actin on the ATPase activity of the myosin and with the precise structural form of the actin-myosin complex, as these are so directly linked to the contraction mechanism in striated muscle. It is difficult to believe that such close homology

would exist unless the same basic mechanism was involved in each case.

Indeed, one can take this argument a stage further and suggest that the actin filament-myosin head interaction was developed as a motile mechanism very early in evolution, and that cells have been using it ever since, with a high degree of conservation of the essential protein interactions involved. As multicellular organisms developed, certain cells specialized in producing more extensive and more powerful movements, and the contractile proteins in them became organized into the larger structures required to integrate the smaller scale motile processes into directed forces. But the same basic molecular mechanism was retained.

In striated muscle, it has been shown that the mechanism depends on the development of a relative shearing force between filaments of actin and myosin. Because so many of the underlying structural and biochemical features of this interaction are shared by the more primitive systems, it seems highly probable, to say the least, that these systems must also operate by an active shearing mechanism, in which sliding forces are developed between polarized actin filaments and some form of myosin assembly. For example, in cases where cytoplasmic streaming is taking place next to a stationary cortical gel layer, one might suppose that actin filaments, attached to the inner cell surface and lying approximately parallel to it, with appropriate structural polarity, generate an active shearing force by their interaction with assemblies of myosin molecules in the more fluid cytoplasm, which is therefore propelled along, carrying with it other cell organelles. If the myosin assemblies, which could be quite small, contained two sets of myosin molecules with opposite polarity (either in the form of bipolar filaments, or perhaps as "face-polar" sheets as described in smooth muscle by Lowy *et al.*⁴⁸ and Small and Squire⁴⁹), then the second set could interact with actin filaments of appropriate polarity in solution, and propel those along too. Alternatively, the location of the actin and myosin assemblies could be interchanged.

Cell organelles could be moved more directly by means of attached actin filaments, which could interact with myosin in large or even very small assemblies and propel themselves along in a manner which I suggested some years ago⁴, or by the interactions described above.

Where a cell, for instance in tissue culture, is moving over a substratum, it is clear that one part must be anchored to the support and that another part of the cell must move relative to that attachment site and form new attachment sites of its own. As attachment sites on and between cells often show filaments trailing back from them, it is natural to suggest that those filaments represent one component of an active shearing system, say, actin, and that the other components are in the cytoplasm and therefore can flow forward over the attached filaments. The filament attachment sites, perhaps analogous to fragments of Z-lines, would pass through the membrane and anchor to the substratum. As the front part of the cell was pushed forward by the internal pressure generated by the cytoplasmic stream, fresh attachment sites for actin filaments might be laid down at the leading edge. As actin filaments polymerized onto these (with appropriate polarity) they would be pulled back by shearing forces developed with the cytoplasm. This might give rise to the appearance of "ruffling" and backwards flow of the unattached membrane of moving cells. When the attachment sites became anchored to the substratum, however, the cell as a whole could then move forward as before over the attachment point (Fig. 3). (See also Bray, *Nature New Biology*, in the press.)

The character of encounters with other cells would depend on whether the external attachment sites on the two interacting cells could attach to each other, or only

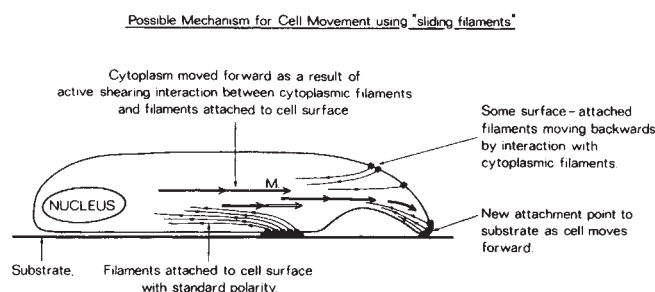


Fig. 3 Diagrammatic representation of a mechanism by which active shearing forces developed between two sets of filaments could produce cytoplasmic streaming and cell movement.

to a third, freely movable site on the opposing membrane. In the latter case, the attachment sites between cells would be drawn to their edge, sustained overlap could not occur, and indeed the entire actin complement of that region of the cell might become tied up in such junctions, leading to "contact inhibition"⁵⁰. In the former, then, overgrowth could occur. Whilst these latter suggestions are entirely speculative, the force of the earlier arguments may make them worth considering and testing.

Another problem, about which there is very little experimental evidence at present, concerns the regulation mechanisms used in non-muscular motile systems. Because activity in muscle is controlled by small changes in calcium concentration, it would be reasonable to look for similar changes, and for the necessary calcium sequestering structures, in other motile systems. Evidence for the presence of elements of the troponin-tropomyosin system in slime mould⁵¹ and in platelets⁵² has recently been described, and evidence for the presence of calcium-sequestering structures has been described in *Spirostomum*⁵³ and *Physarum*⁵⁴.

Finally, I should mention that there are now numerous other instances where actin-like filaments have been implicated in cell movement, though the evidence does not have the decisive character of the examples I have given earlier. In many of these, particularly in the cases of various types of morphogenetic movement, biochemical identification of actin will be difficult. There are, however, several instances where actin has been identified by arrowhead formation with HMM⁵⁵. This is a very specific test for an actin-like protein (though not necessarily for its location in polymerized form unless actin-like filaments can be seen in the same position in untreated material). For example, Perry *et al.*⁵⁶ showed that a ring of filaments, just below the surface of the cleavage furrow at the newt egg, would bind HMM to give arrowhead complexes. This ring of filaments has been described by several workers on jellyfish eggs^{57,58}, and also on the eggs of a polychaete worm⁵⁸ and on the ciliate *Nassula*⁵⁹ as a contractile structure which, by decreasing its diameter, is responsible for at least a large part of the cleavage process. Two opposing sets of actin filament, linked by myosin, could have this property.

Tilney and Mooseker⁶⁰ have shown that filaments located in the microvilli forming the brush border of epithelial cells of chicken intestine also give arrowheads with HMM, behave like actin on SDS gels and, especially interestingly, appear clearly to be anchored to the cell membrane; thus they may aid transport by some kind of pumping action.

In summary then, there seems to be good evidence that several types of cell movement are brought about by molecular mechanisms which use proteins very similar to actin and myosin in muscle. A strong presumption exists, therefore, that active sliding or shearing mechanisms are involved. This possibility needs to be explored in each case, especially by structural and mechanical studies on the detailed processes involved.

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Transformation of Mammalian Cells *in vitro* by Low Doses of X-rays

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X-irradiation of hamster embryo cells in culture, followed by cloning of the cells, was used to obtain a dose-response curve in terms of cell transformation. Transformations were observed for doses as low as 1 rad.

PREVIOUS reports have described the induction of malignant cell transformation by a dose of 300 rad of X-rays in short term cultures of hamster embryonic cells¹⁻⁴. The conditions required for the fixation of this transforming event as a hereditary property of the cells have also been reported^{1,3,4}, as well as some of the *in vitro* characteristics of the X-ray transformed cells¹⁻⁶ and their capacity to induce tumours *in vivo*^{1,2}. Recently, some of these investigations have been repeated using established mouse cell lines⁷. A comprehensive study of the carcinogenic action of radiation must include a knowledge, at the cellular level, of the relationship between the absorbed dose and the probability of neoplastic conversion.

This report describes experiments designed to elucidate the dose-response relationship over a range of doses from 600 rad down to 1 rad.

Experimental Techniques

Minced midterm whole embryos from golden hamsters were used as the source of normal cells. Primary cultures were established by progressive dissociation of the minced fresh tissue⁸. Cells (10^7) were seeded into each 100-mm Petri dish (Falcon Co.) and incubated at 37° C in a humidified incubator with 5% CO₂ in air. Three-day-old primary cultures were prepared into a cell suspension by trypsinization, and cloned into 60-mm Petri dishes on X-irradiated (4,000 rad) feeder cells of the same type⁹. Seeding levels ranged from two thousand to ten thousand cells, depending on the dose to be administered, thus allowing for cell killing.

In the experiments to study transformations, the cells were irradiated at room temperature, 24 h after seeding, with a range of doses from 1 to 600 rad. The source of X-rays was a 210 kV Westinghouse constant potential generator, with added filters of 0.5 mm of copper and 1 mm of aluminium. For the higher doses (75–600 rad) a treatment distance of 50 cm was used, corresponding to a dose-rate of 70.6 rad min⁻¹. For the lower doses (1–10 rad) a longer treatment distance of 250 cm was used at which the dose-rate was 4.25 rad min⁻¹; the lower dose-rate was used to avoid shutter errors associated with very short exposure times. Doses were measured with a Victoreen R-meter, calibrated at the Bureau of Standards.

After a post-irradiation incubation period of 9 d the clones

were fixed and stained¹. A differential count was made of normal and transformed clones, the latter being identified by piled-up morphology, random cell orientation and loss of contact inhibition, characteristics not seen in the untreated control cultures¹⁻⁴.

In several experiments, transformed clones were isolated and grown into mass cultures. The ability of the cells to form clones in semi-soft agar^{10,11} and their agglutinability by concanavalin A and wheat germ agglutinin (provided by M. M.

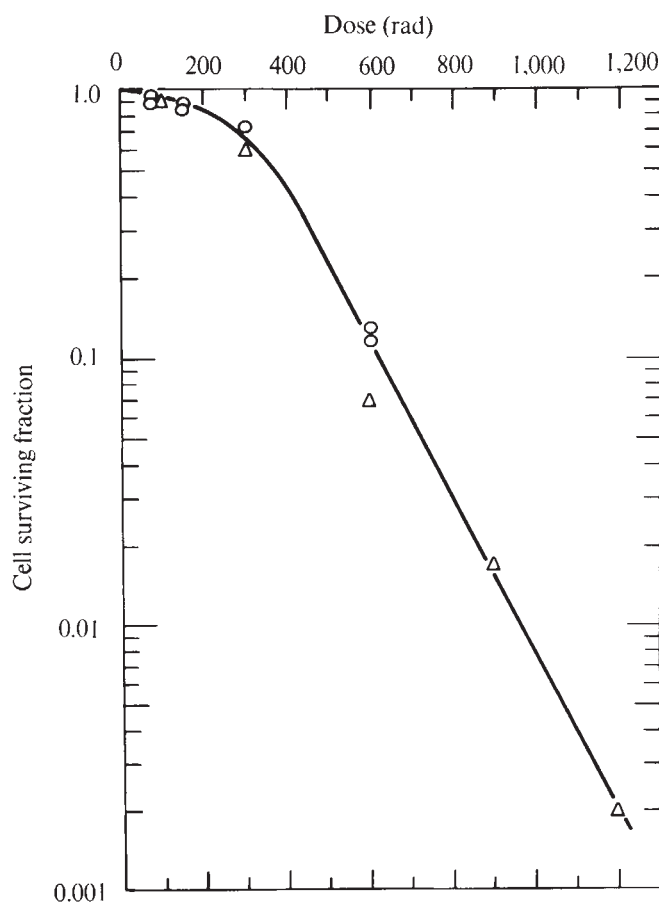


Fig. 1 Survival of reproductive integrity of hamster embryo cells as a function of X-ray dose. Circles describe points calculated from the transformation experiments (Table 1). Triangles describe points established from a separate cell survival experiment (see text). N , The extrapolation number, defined as the point in the surviving fraction axis to which the straight portion of the surviving curve extrapolates, = 6. D_{01} , The dose required to reduce the population to a fraction of 37% (used as a measure of the slope in the linear portion of the survival curve), = 147 rad.

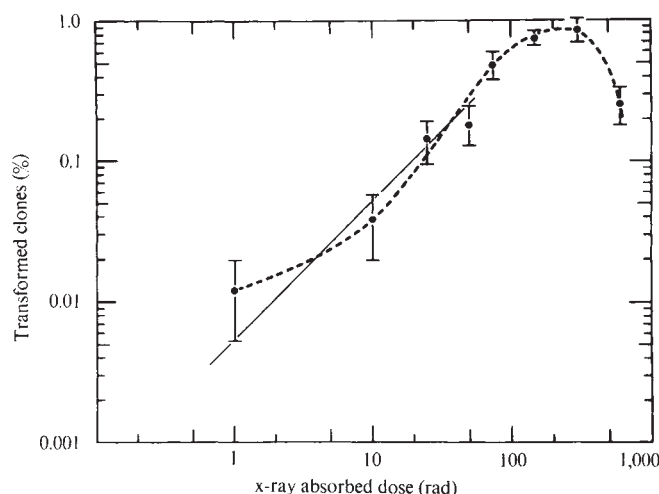


Fig. 2 Incidence of hamster embryo cell transformation following exposure *in vitro* to X-irradiation. For doses at which more than one experiment was performed, the data were pooled; the mean value together with the standard deviation is plotted in the figure (see Table 1 for results of individual experiments). The broken line is drawn by eye to the mean data points; the full line has a slope of +1 and passes through the error bars of each datum point.

Burger)^{6,12,13} were used as further criteria for the transformed nature of the cells as compared with control cultures.

Dose-Response Relationship for Transformation

The data for cell survival are shown in Fig. 1, in which dose is plotted on a linear scale against surviving fraction on a logarithmic scale. The shape of the survival curve is characteristic of that commonly observed for mammalian cells exposed to sparsely ionizing radiations, such as X-rays¹⁴. At low doses there is a broad initial shoulder, implying that X-rays are relatively inefficient at killing cells in this region; for larger doses the survival curve becomes a straight line in this semi-logarithmic plot, implying that over this dose range cell survival is an exponential function of dose.

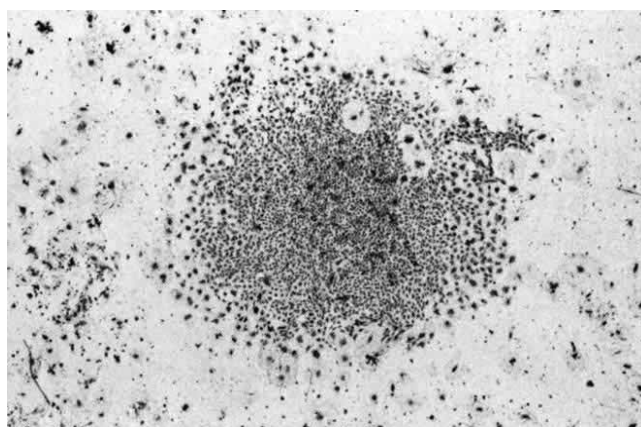


Fig. 3 A 9-d-old clone of normal embryo cells. Giemsa stain $\times 11$.

The dose-response data for transformation are shown in Table 1 and Fig. 2, in which dose is plotted against transformation (%) on a double logarithmic plot. The percentage of cells transformed increases as the dose is increased up to a plateau of approximately 1%, which corresponds to doses of between 150 and 300 rad. A further increase in dose results in a marked decrease in the proportion of cells transformed. Photographs of representative clones, both normal and transformed, are shown in Figs 3-6.

From an examination of the stained cultures it was apparent that the dominant type of transformed clone consisted of cells which were piled up, one on another, having lost contact inhibition¹⁵, but were less randomly orientated than hamster cells transformed by some oncogenic viruses or chemical agents¹. This morphology has also been observed when tumours induced by X-ray transformed cells were cultured and cloned *in vitro* (C. B., unpublished). Transformed clones of epithelioid morphology^{8,13} were found at a very low frequency. In contrast to controls, the X-ray transformed cells derived from all of the ten isolated clones were agglutinable by 50 $\mu\text{g ml}^{-1}$ of concanavalin A or wheat germ agglutinin. The ability to form colonies in 0.33% agar was limited to the cells of the two isolated transformed clones of epithelioid morphology.

Table 1 Neoplastic Cell Transformation of Hamster Embryo Cells following X-irradiation *in vitro*

Experiment No.	Control No. of clones counted	Control Plating* efficiency	No. of transformed clones	Dose (rad)	Irradiated No. of clones counted	Irradiated No. of transformed clones	Transformed† clones (%)	Surviving‡ fraction
1	1,600	1.6	0	600	920	2	0.22	0.12
2	1,002	2.2	0	600	3,020	8	0.27	0.13
3	1,012	1.3	0	300	1,130	8	0.71	0.75
4	1,220	1.6	0	300	1,960	18	0.92	0.75
5	2,500	4.5	0	150	6,200	44	0.71	0.89
6	4,020	2.1	0	150	3,268	25	0.77	0.86
7	1,620	2.0	0	75	2,132	11	0.52	0.92
8	4,100	1.2	0	75	2,200	10	0.46	0.95
9	1,025	1.05	0	50	5,402	10	0.185	0.95
9	1,025	1.05	0	25	5,503	8	0.145	1.00
6				10	10,200	4	0.039	1.00
8				1	6,800	1	0.015	1.00
10	9,600	2.5	0	1	9,200	1	0.011	1.00
10	7,200	1.9	0	1	7,900	1	0.013	1.00

* Plating efficiency (P.E.) = $\frac{\text{Number of cells plated}}{\text{Number of clones counted}} \times 100$

† Cell transformation (%) = $\frac{\text{Number of transformed clones}}{\text{Number of clones counted}} \times 100$

‡ Surviving fraction = $\frac{\text{Number of cells plated}}{\text{Number of clones counted} \times \text{P.E.}}$

A preliminary study of the karyotypes of the X-ray transformed cells using the Giemsa banding method¹⁶ indicates that the cells tend to be aneuploid but there are, apparently, no gross chromosomal aberrations. Experiments kindly conducted by Dr I. B. Weinstein, using reported techniques¹⁷, demonstrated that there were no C-type virus particles present in X-ray transformed cells which had been proved to be able to induce tumours¹.

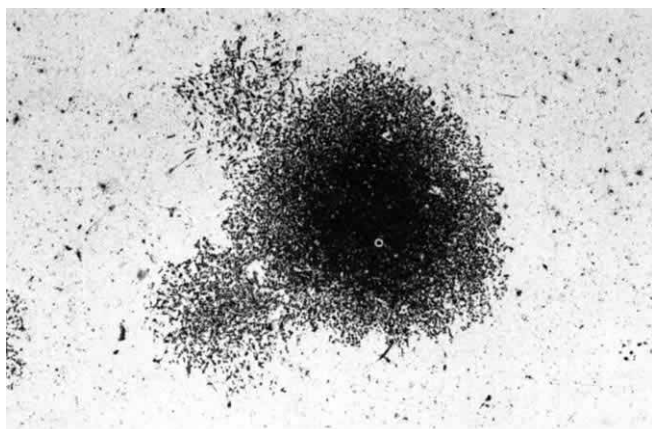


Fig. 4 A 9-d-old clone of hamster embryo cells transformed *in vitro* by 300 rad of X-irradiation. Giemsa $\times 11$.

These results demonstrate that neoplastic transformation can be induced in single cells with doses of X-rays as low as 1 rad. At any of the doses used, the fraction of cells killed is small, and this, coupled with the fact that no transformed clones have been observed in control cultures, strongly supports the view that transformation is directly induced by X-irradiation, and is not a result of a selection of spontaneously occurring transformed cells.

Dose-response relationships for the induction of neoplasms by ionizing radiation have been studied and discussed previously, using human and animal data¹⁸⁻²¹. The shape of the dose-response curve obtained in the present *in vitro* experiments is not unlike that characteristic of *in vivo* data. It consists of three portions; an ascending limb, a plateau and a descending limb.

In the *in vivo* situation there are insuperable difficulties involved in obtaining significant data for low doses of radiation in the range of 1-25 rad, where tumour incidence is low and inordinately large numbers of animals would be required to detect it. Consequently there has been much speculation concerning the actual shape of the ascending limb of the curve. Linear extrapolation from higher doses, on the one hand, has been the conservative approach, while the existence of a threshold dose below which there is no effect cannot be ruled out^{22,23}. One of the principal assets of the *in vitro* system is that a very low frequency of transformation can be detected by irradiating large numbers of isolated single cells and scoring thousands of clones. A cell which has received a "hit" at a crucial site and been converted to a neoplastic state can replicate freely and thereby fix this event as a hereditary property², and grow into a transformed clone.

If a curve is naively drawn through the data points in Fig. 2, it would have a slope of less than unity for doses between 1 and 10 rad, which on this double logarithmic plot would imply that the transformation rate increases with a power of the dose that is less than one. This kind of dependence has been observed for the induction of mammary neoplasms by neutrons.

Rossi and Kellerer²⁴ have pointed out that in that instance it was necessary to assume a multicellular mechanism of tumour induction because the number of neutron secondaries per cell was considerably less than one. In our experiments the number of X-ray secondaries (electrons) per cell, or even per cell nucleus, is considerably larger than one and the shallow slope which appears to fit the data best may be due to a range of susceptibility among the cells. But the confidence limits on the data points, particularly at the lower doses, are such that one can draw a line with slope +1 that does not fall outside the error bars of any of the points. A linear dependence of induction on dose therefore cannot be ruled out.

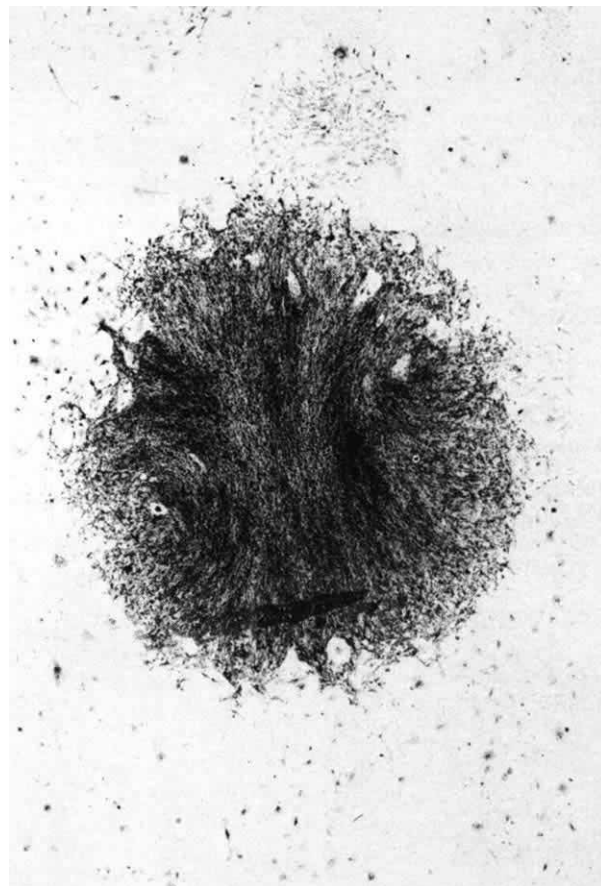


Fig. 5 A 9-d-old clone of hamster embryo cells transformed *in vitro* by 1 rad of X-irradiation. Note the random orientation on the circumference. Giemsa $\times 15$.

The plateau between about 150 and 300 rad closely resembles that often reported for *in vivo* experiments²⁰. For a change in dose by a factor of two, the frequency of neoplastic conversion, which is about 1%, remains essentially unaltered. A further increase in dose above 300 rad results in a marked decrease in the frequency of transformation. Similar observations made in humans and animals¹⁸⁻²¹ have resulted in speculative interpretations suggesting that the overall high cell killing occurring in these multi-hit dose ranges, and reflected in the survival curves, is responsible for the sharp decline in capacity to induce tumours.

Our data support the idea that above about 300 rad, cell reproductive death²⁵ begins to be an important factor; the percentage of transformed clones declines above 300 rad in spite of the fact that they are scored from amongst the survivors of the irradiated population, implying that there is a preferential killing of potentially transformed cells. If normal and poten-

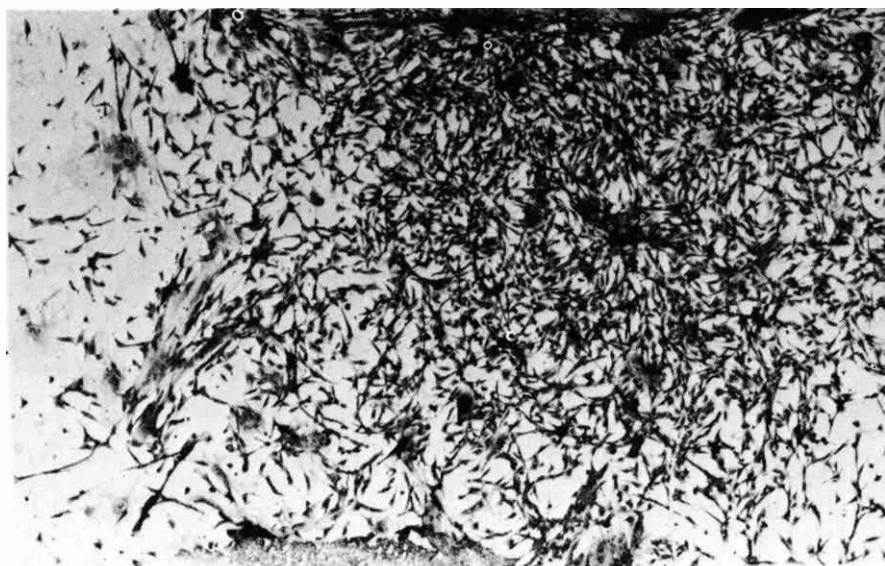


Fig. 6 A section of the circumference of the clone in Fig. 5. Giemsa $\times 45$.

tially transformed cells were equally sensitive to radiation, then a decline in the proportion of transformed cells at higher doses would not be expected. These results support the ideas proposed by Gray²¹ inasmuch as they suggest that cells that have been altered by the absorption of radiant energy and are destined to become transformed cells, are more susceptible to being killed by the accumulation of additional damage at higher doses. In other words, many of the potentially transformed cells never have the chance to form a colony because they die a reproductive death due to the extra damage produced by the additional "hits" received at larger doses.

One of the drawbacks of this technique stems from the use of the whole embryo, as a result of which there is a mixture of many cell types. Furthermore, the cell cultures are asynchronous at the time of irradiation, so not all cells are equally at risk. Differences in cell sensitivity to low doses of radiation at various stages of the cell cycle have been reported²⁵⁻²⁷.

Our preliminary karyotype analysis of the cells transformed by a dose of 300 rad of X-rays revealed no gross chromosomal aberrations within the limitations of the banding technique used¹⁶. The event that leads to transformation, however, may well be a subtle genetic structural alteration, such as a gene mutation, which results in the loss of control of cell replication and alteration in the cell surface.

While all the X-ray transformed cells exhibited a microstructural surface change characteristic of the neoplastic state and detectable by plant agglutinins^{6,12,13}, not all of them form colonies in semisoft agar. As mentioned previously, most of the X-ray transformed cells which are fibroblast-like, and the cells grown from tumours induced by these transformed cells, are not as randomly oriented as those transformed by oncogenic viruses or chemicals. This suggests that these cells have not completely lost "anchorage dependence"²⁸ which is a requirement for growth in a semi-solid medium such as agar. The cell type which did grow in agar was of epithelioid origin with different growth patterns. They multilayered as rounded cells exhibiting very little adhesion to one another and to the surface on which they were grown.

Some types of leukaemia induced by X-irradiation have been found to be associated with the presence of viruses, implying an indirect action of the ionizing radiation^{20,29}. Our findings indicate the absence of C-type virus particles in transformed cells which on injection into newborn hamsters give rise to fibrosarcomas.

In vivo studies on neoplasias induced by X-rays have stressed the wide range of tumour types which arise¹⁸⁻²¹. The fact that neoplastic transformation induced *in vitro* by X-rays

cell, together with a striking similarity between the *in vivo* and *in vitro* dose-response relationships, make this system suitable for further studies on the carcinogenic action of X-rays and other ionizing radiations present and increasing in our environment.

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Seafloor Spreading in the Tasman Sea

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Magnetic and seismic studies in the Central Tasman Sea show conclusively that it evolved by a process of seafloor spreading between 60 and 80 m.y. BP, suggesting that other marginal basins may have a similar history. A reconstruction of Australia, the Lord Howe Rise, New Zealand and Antarctica based on these data is presented and discussed.

TECTONIC theory can account for the first-order features of the principal ocean basins, but the origin of the marginal basins of the western Pacific is still in doubt. The crust underlying the marginal basins is similar in thickness and compressional wave velocity characteristics to that of the major ocean basins^{1,2}, but the depth of the seafloor in these marginal seas is somewhat variable, chiefly because of sediments on the underlying crust. Karig³ concluded that marginal basins were formed by crustal extensions and that their genesis was related to the proximal arc-trench systems and associated seismic Benioff zones. An alternative proposal calls for injection of mantle material along rifts within or behind an island arc, thus producing a "half-cell" style of seafloor spreading⁴.

The Tasman Basin, the West Philippine Basin, and South Fiji Basin were classified by Karig as inactive, marginal basins with normal heat flow³. The normal heat flow was taken as evidence that the basins had been devoid of major extensional tectonism for the past 20 to 40 m.y. Karig³ goes on to say "it would be hazardous to assume the presence of identifiable linear magnetic anomalies in marginal basins until at least one diagnostic survey confirms that assumption".

Here we report new data from the Tasman Sea, which reliably confirm identifiable linear magnetic anomalies which can be recognized as part of the worldwide pattern of Cretaceous spreading lineations⁵. These data, together with seismic reflexion profiling and detailed bathymetric studies, provide conclusive evidence that the Central Tasman Sea has evolved through a process of seafloor spreading similar to that taking place at the seismically active crests of the mid-oceanic ridges. These processes have been dormant within the Central Tasman Basin for about the past 60 m.y. and the area is now aseismic.

The Tasman Sea is bounded on the west by the Australian

Tasman Rise system; on the east and northeast by the Lord Howe Rise, New Zealand and the Macquarie Ridge Complex (Fig. 1a). The southern boundary is poorly defined but is often taken to be the crest of the South-east Indian Ridge System. Magnetic lineations have been mapped previously in the most southern portion of the Tasman Sea and have been related directly to seafloor spreading about the South-east Indian Ridge and to the migration of Australia away from the Antarctic continent commencing about 50 to 60 m.y. ago⁶. Weissel and Hayes⁶ and Hayes and Conolly⁷ suggested that a morphologic and crustal boundary separated the South Tasman Sea from the Central Tasman Sea along a line extending northeast from the South Tasman Rise to the Resolution Ridge near Fiordland, New Zealand (Fig. 3). We have recognized for some time a major contrast in the sediment disposition of the Tasman seafloor in the area to the north and south of this hypothetical boundary.

Ringis⁸ has correlated northwesterly trending magnetic lineations offset along northeast-southwest lines in the Central Tasman Sea, and these lineations were later confirmed by USNS Eltanin^{9,10}. Griffiths¹¹ speculated on the origin of the Tasman Sea by examining the "geological constraints" imposed by the reconstruction of Australia to Antarctica and their interrelation to New Zealand, the Lord Howe Rise and the Campbell Plateau. Griffiths¹¹ drew on the preliminary data of Ringis⁸ to conclude, incorrectly, that the Central Tasman Sea was tectonically active until late Miocene, about 10 m.y. ago. Van der Linden proposed that the Dampier Ridge is a dormant site of seafloor spreading¹², and it has also been suggested that the Tasmanid guyots mark the northward migration of the Tasman Basin across a fixed mantle hot spot, thus recording the motion of Australia away from Antarctica¹³. The inferred age of the Tasman Basin based on the depth of the top of the oceanic crust and simple thermal cooling models was 90 ± 40 m.y. (ref. 13). A cursory examination of widely spaced east-west aeromagnetic profiles between Australia and the Lord Howe Rise led to the early but incorrect conclusion that there were no lineated magnetic anomalies within the Tasman Sea¹⁴.

In Fig. 1b we show the tracks along which geophysical data were available to us. Most of these lines were run by USNS Eltanin (47A). The dotted lines represent aeromagnetic flight lines along which magnetic data are the only geophysical parameter available. The other data were collected along ship tracks and most tracks include precision depth recording, seismic reflexion profiling, total intensity magnetic anomaly observations and, in many cases, gravity observations.

although our discussion takes cognizance of the much larger total collection of data available for this area.

Magnetic Anomalies

In Fig. 2 selected total intensity magnetic anomaly profiles are aligned on an axis of symmetry and are arranged roughly from north to south, showing not only the correlation of anomaly lineations in this area, but also identifying the individual lineations as a portion of the worldwide Cainozoic

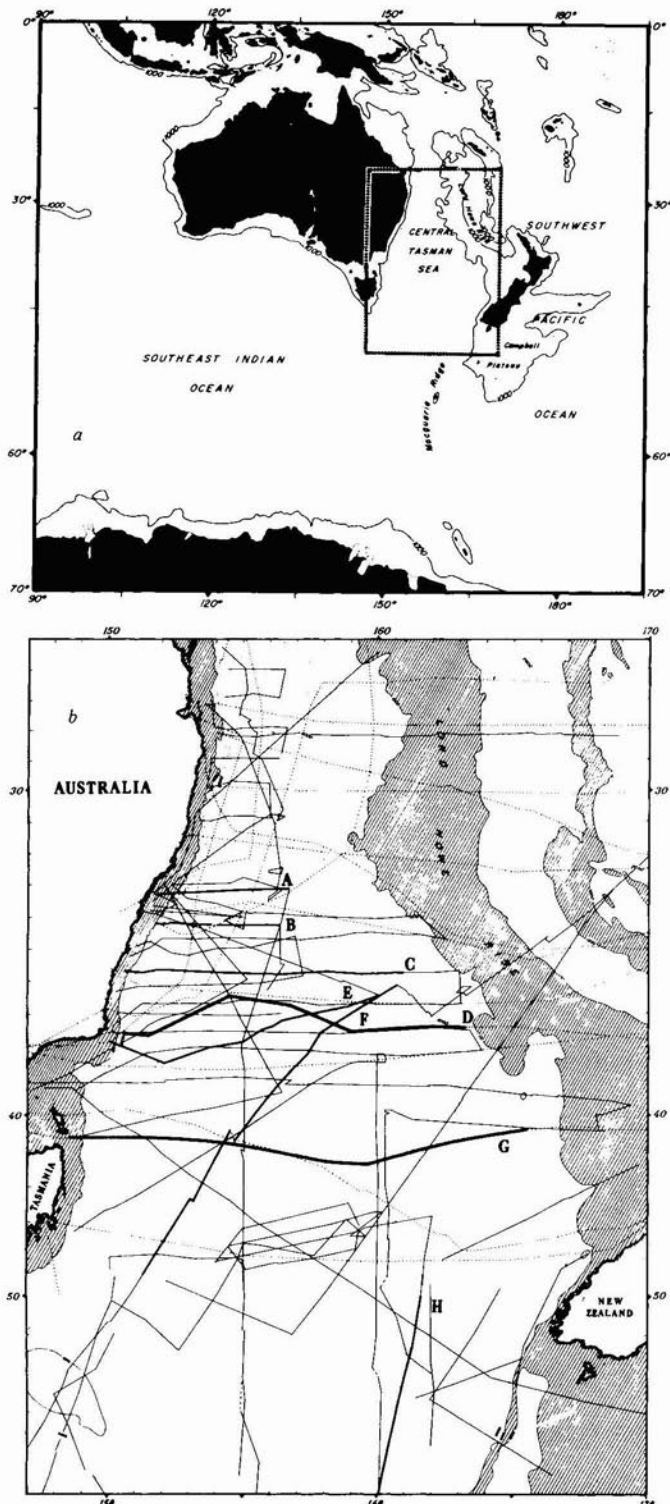


Fig. 1 *a*, Area of study in relation to regional geography. Rectangle encloses area detailed in *b*. *b*, Ship tracks (—) and aeromagnetic flight lines (...) along which geophysical data were examined in this study. Letters key selected profiles shown in Figs. 2 and 6.

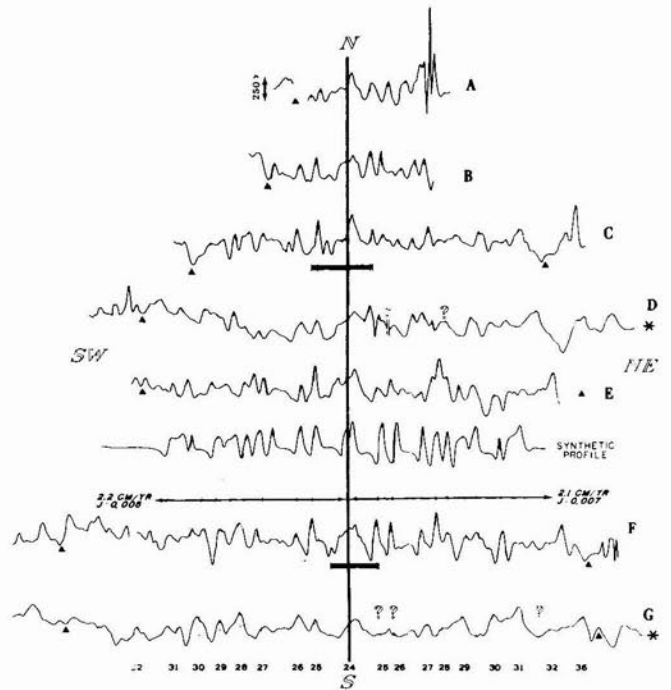


Fig. 2 Selected total intensity magnetic anomaly profiles. Asterisks denote aeromagnetic data. See text for further explanation. Profile locations as Fig. 1*b*.

pattern⁵. Two of the seven observed profiles shown are aeromagnetic profiles (marked by asterisks) and the somewhat longer apparent wavelengths and attenuated amplitudes are attributed to the increased distance from source to detector compared with anomalies measured at sea level. One synthetic anomaly profile, computed using standard techniques^{15,16}, is shown near the centre of the figure. Many synthetic profiles were computed (using different natural remanent magnetization parameters) and the profile shown is an example of the range of "optimum" or representative fits for purposes of identifying the observed lineations.

Although issue may be taken with any single correlation or identification shown in Fig. 2, when one considers the entire pattern and also the many additional profiles that were available, the overall correlations, the lineation ages, and the magnetic fabric are all considered to be very well defined. The inverted carats mark the approximate position of the abrupt increase in slope along the margins of Australia and the Lord Howe Rise. The significance of the slight differences in spreading rates indicated for opposite sides of the synthetic profile will be discussed in a more complete study. The presence or absence of small offsets in the lineation pattern cannot be proven with the reconnaissance data. Such undetected offsets would give rise to small apparent differences in the inferred spreading rates and will introduce complexities in unequivocally identifying all of the anomalies on any particular profile.

The heavy bars shown below profiles C and F mark the position near the crestal zone of the buried basement ridges discussed in the next section. Figure 3 synthesizes the overall magnetic lineations fabric using all the available magnetic, seismic, and topographic data represented by the collective tracks shown in Fig. 1*b*.

Seismic Data

Seismic reflexion profiler data reveal a distinct basement ridge whose axis is coincident with the centre of symmetry of the magnetic lineation pattern previously identified as anomaly 24 (age ~60 m.y. BP). The basement morphology is almost entirely blanketed by a thick cover (>1 s two way reflexion

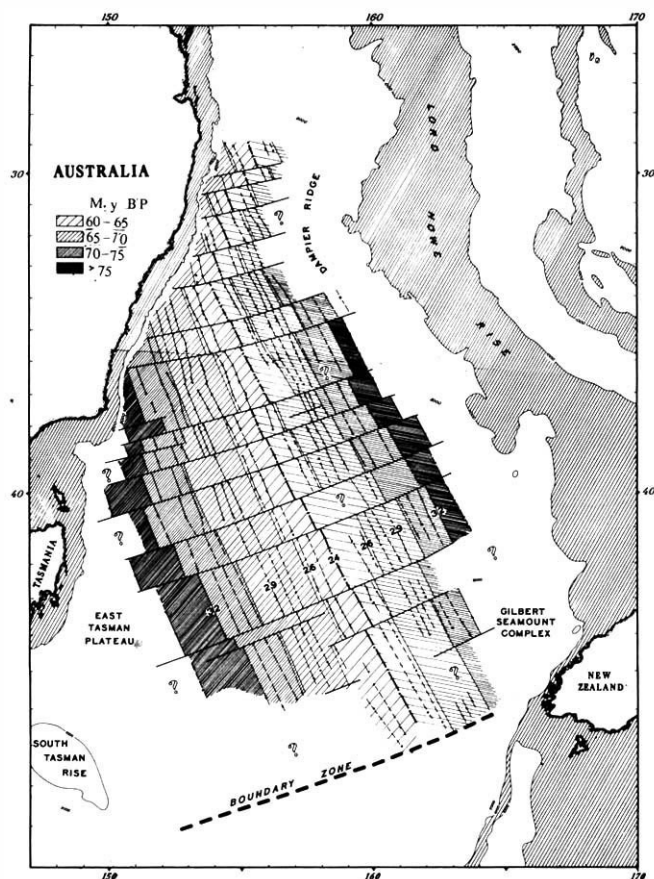


Fig. 3 Magnetic lineations, fracture zones and age of the basement of the Central Tasman Sea. Anomaly identifications (located by dots) and ages according to the Heirtzler *et al.* system¹⁵.

time) of acoustically transparent sediments, which thin subtly near the crest of the buried ridge and in general smooth out both the regional and local relief of the underlying basement surfaces. Some of the larger basement peaks are still expressed (through the semi-conformable sediment layer) in the topography of the seafloor. Only one sub-bottom reflector can be traced over any significant distance, but it is not pronounced, indicating that it represents only a slight change in the physical properties (acoustic impedance) and the inferred sedimentary regime. Adjacent to the Australian continental margin the total sediment thickness is much greater than elsewhere, the primary difference here being the presence of about 0.75 km of layered, flat-lying, presumably interbedded turbidites and

hemipelagic sediments. The total thickness of sediments beneath the Tasman abyssal plain was not recorded with our seismic reflexion profiling technique.

The "typical" ridge morphology consisting of a distinct crest, axial rift, and gently sloping symmetric flanks is best developed through the northern half of the Central Tasman Sea and is well illustrated in profile X, Fig. 4, which is nearly orthogonal to the trend of the buried basement ridge. Further to the south, the crest of the ridge is less pronounced (Fig. 4 Y, Z) although still recognizable. Small offset fractures probably transect each of the profiles in Fig. 4.

Using simplified thermal models, a theoretical-empirical relationship has been proposed between the age of the basement crust and its depth below sea level¹⁶ which predicts elevations at the crest of $5,200 \pm m$ and $5,500 \pm m$ at the distant portion of the flanks. The observed depths at the crest range from about $4,500 \pm m$ in the north to about $5,200 \pm m$ in the south. The flanks near Lord Howe Rise are near $5,500 \pm m$ while those near Australia are greater than $6,000 \pm m$. These results are in fairly good agreement with the models¹⁶ although the elevations of these models are not too sensitive to large age differences for oceanic crust older than about 60 m.y. The greater depth to basement observed along the Australian margin is attributed in part to sediment loading and flexure of the crust, but we cannot exclude the possibility that some limited crustal subduction occurred along this margin while the Central Tasman Sea was actively spreading.

The trend of the buried basement ridge and the magnetic lineation trends are internally consistent, and within the limits of resolution, the axis of symmetry within the magnetic pattern and the axis of the basement ridge are coincident (Fig. 5). The trend of the ridge axis and the magnetic lineation pattern and offsets in the pattern are all oblique to the regional trend of the Victoria-New South Wales portion of the Australian continental margin. The extinct ridge axis and central anomaly (24, ~60 m.y. BP) nearly intersect the Australian continent in the northern portion of the Tasman Sea, just west of the Dampier Ridge (Fig. 3). In addition, the oldest crust recognized in this region is only about 70 m.y. old, or more than 10 m.y. younger than the oldest crust recognized further to the south. Either spreading began somewhat later or was much slower in the northern area than in the southern area, implying a major shear zone between the two, or spreading in both areas was synchronous but the magnetic lineation evidence for the older crust in the north was obliterated by subduction to the west and/or later volcanism on the east. We find no evidence to substantiate the proposal that the Dampier Ridge is an extinct mid-ocean ridge¹⁷. It seems that the Dampier Ridge is a somewhat younger feature than the adjacent seafloor even though extrapolation of crustal ages to the east of the extinct spreading axis would imply it was an older feature.

A major submarine canyon located just east of Bass Strait coincides with the south-westerly extension of one of the larger offset fracture zones recognized in this area. The offset of the isobaths defining the continental slope are right lateral in sense, consistent with the offset of the ridge axis across the same fracture zone (Fig. 3). This canyon and other similar topographic irregularities along the steep slopes of the Australian margin and western slope of the Lord Howe Rise are probably expressions of fracture zone offsets in the initial pattern of rifting. Similarly, concentrations of Cretaceous and Early Cainozoic volcanism near the Australian coast and the locations of large seamounts are probably controlled by the relict lines of crustal weakness defined by fracture zone offsets in the initial extensional rift.

The southern limit of the late Cretaceous to Palaeocene Tasman Sea opening is now rather clearly defined both on the basis of reflexion profiling data and magnetics data. Figure 6 shows the dramatic contrast in the nature and thickness of the sediment blanket, the depth to the basement layer, and the apparent relief of the basement on opposite sides of the boundary zone. The zone trends east-northeast parallel to

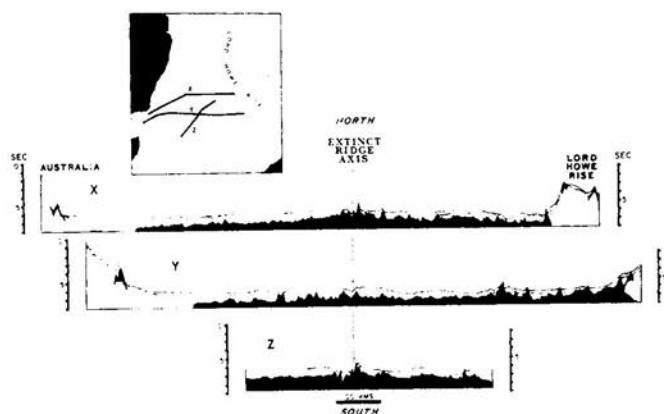


Fig. 4 Line drawings of seismic reflexion profiles. Black areas represent oceanic basement (layer 2; $V_p \approx 5 \text{ km s}^{-1}$).

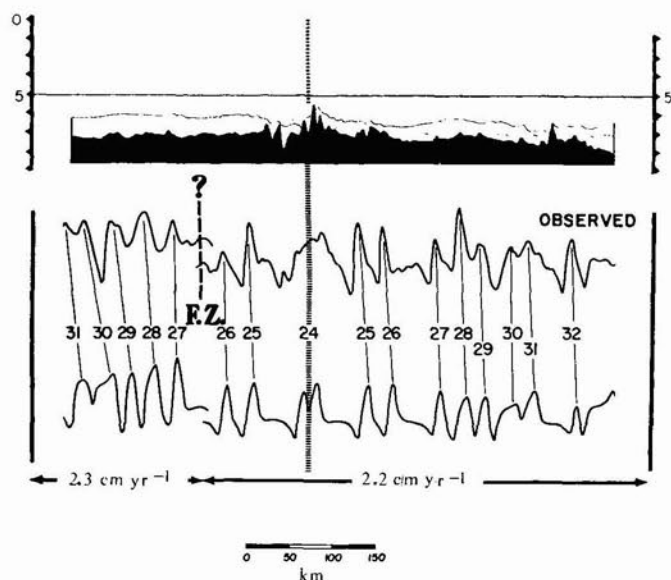


Fig. 5 Profile Z of Fig. 4 together with observed total intensity magnetic anomaly and two dimensional synthetic anomaly, computed using the geomagnetic time scale of Heirtzler *et al.*¹⁵. We note the coincidence of the axial anomaly (24) and the crest of the buried ridge.

the inferred fracture zones to the north and is expressed as the Resolution Ridge near 46° S, 165° E and extends to the southern limit of the South Tasman Rise near 49.5° S, 152° E. The strike of the boundary zone is also subparallel to the major strike of the Alpine Fault. The tectonic and lineation fabrics north and south of this zone are approximately orthogonal to one another.

This boundary zone must have been the locus of major shearing from 80 to 60 m.y. BP and may have corresponded to the initiation of transcurrent movements along the Alpine Fault or similar fault zones. The orientation and location of the shear zone boundary undoubtedly played a major role in determining the subsequent orientation and locus of the younger rift system (now a part of the Southeast Indian Ridge) along a line of previously established crustal weakness.

Continental Reconstructions

By a trial and error rotation to near coincidence of corresponding magnetic anomaly lineations located on opposite sides of the extinct ridge axis, one can determine a pole and finite rotation angle that best describes the total average relative motion for discrete time intervals^{6,18}. If small time intervals are examined in sequence, backward in time, one can approximate the paths of relative motion between the two spreading plates. If, however, one is only concerned with the relative configuration of plates at any given moment of time, a similar procedure may be used but the pole and angle of rotation deduced will not in general define the relative motion for a long time interval¹⁸. The pole and rotation angle are simply a mathematical convenience in defining the total relative motion and the actual motion could be the resultant of many finite rotations about various poles, but which must sum to the same total rotation about the single pole. Here we simply consider the pre-rifted configuration of the area by determining the pole which brings into closest coincidence the oldest anomaly lineations (36, ~80 m.y. BP, ref. 19) recognized there. Anomaly 36 lies close to the continental edges of east Australia and the Lord Howe Rise as inferred by the positions of the continental slopes. Because of the well developed magnetic pattern, there is not much uncertainty in the pre-rifted configuration of these two continental elements. Using similar techniques the pre-rifted configuration of Australia and Antarctica can be rather

precisely determined⁶. The magnetic lineation pattern between the Campbell Plateau and West Antarctica is defined only to the north of the Pacific–Antarctic Ridge; hence the technique cannot be used to reconstruct these two continental elements. An additional complication is introduced by the fact that earthquake epicentres indicate an active plate boundary between the Lord Howe Rise and the Campbell Plateau that is presently defined by the Alpine Fault and the Macquarie Ridge Complex. The nature of this boundary during the late Cretaceous and Cainozoic is in doubt^{20–21}. Both of these questions can be investigated by utilizing the constraints imposed by closing up the Central Tasman Sea and the Southeast Indian Ocean as dictated by the magnetic and morphological fabric of these areas.

Figure 7 shows the reconstruction to approximately 80 m.y. BP. The morphological “fits” are far from perfect and significant gaps exist within the incipient Tasman Sea. More important, several large morphological overlaps exist which must be explained if the reconstructions are to be considered valid. The seriousness of the overlaps depends on the issue of the continental versus oceanic nature of the overlapping segments. Continental crust overlapping continental crust such as the case of the Campbell Plateau onto Marie Byrd Land is entirely unacceptable and must be eliminated by hypothesizing additional tectonic motions. In Fig. 7 this particular overlap has been minimized by taking into account the known left-lateral, strike-slip motion across the Alpine Fault of ~480 km which is generally agreed to have occurred in post-Early Cretaceous²¹. The total area of “continental” overlap would be much greater if the Alpine Fault movement had not been reconstituted.

The Dampier Ridge overlaps the area of continental Queensland–New South Wales, Australia. If our reconstruction is correct, it could not have been formed prior to the opening of this area to the extent that the ridge could be accommodated, and we therefore conclude that the most likely mode of formation was by oceanic volcanism subsequent to the initial opening of the Central Tasman Sea, thus accounting for the overlap.

The only other areas of serious overlap are the South Tasman Rise (a presumed extension of the Australia–Tasmania continental block) with the Iselin Plateau (an extension of the Ross shelf, also presumed to be underlain by continental type crust). The continental versus oceanic nature of these features is unconfirmed but will be the subject of two DSDP sites to be drilled during the first two legs of Antarctic drilling²³. The very shallow depth to high velocity basement rocks at both sites suggests they are partially founded continental blocks, in which case their overlap is unacceptable.

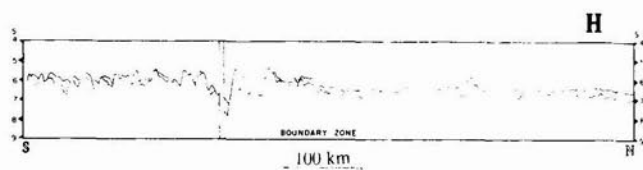


Fig. 6 Line drawing of seismic reflexion profile across the boundary between the Central Tasman Sea and the South Tasman Sea. We note the contrast in sediment disposition. See Fig. 1b for profile location.

In order to reconcile the unacceptable overlaps, one must hypothesize plate motions in addition to those in Fig. 7. Because the reconstructions of Australia to Antarctica and of Lord Howe Rise–New Zealand to Australia are considered now to be well determined, we believe the “unknown” plate motions required for an internally consistent picture must either be accounted for by motion between the Lord Howe Rise and the Campbell Plateau or by motion between East Antarctica and West Antarctica.

The total offset of the axis of the New Zealand geosyncline is about 1,200 km with 480 km accounted for by the Alpine

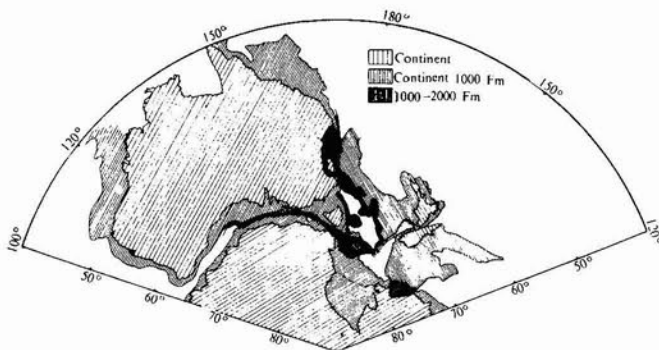


Fig. 7 Reconstruction of Lord Howe Rise, Australia, and Antarctica before about 80 m.y. BP, based on observed magnetic anomaly lineations. The black areas represent regions where there is an overlap onto known continental fragments. See text for detailed discussion.

Fault¹¹. Only the Alpine Fault movement has been accounted for in Fig. 7. If the apparent bending and displacement of the geosynclinal axis does in effect represent an additional right lateral offset of about 700 km, this motion would be more than enough to account for the Campbell Plateau to Marie Byrd Land overlap. But this motion would still not reconcile the overlap of the Tasman Rise with the Iselin Plateau. An alternative explanation is a post-Late Cretaceous relative motion between East and West Antarctica. If West Antarctica were to be moved in a right lateral sense to a hypothetical Early Cretaceous position along a line that passes west of the Iselin Plateau, then all of the serious overlaps of Fig. 7 can be reconciled. East and West Antarctica are quite different in their gross geology²⁴⁻²⁶, and a major morphological and structural boundary separates the two provinces along a line subparallel to and east of the Transantarctic Mountains. The genesis of this structural zone is entirely speculative although a portion of the area, the Bentley subglacial trench, is characterized by basement rocks that presently lie well below sea level²⁶.

We favour motion between East and West Antarctica because it allows for the reconciliation of two areas of major overlap and because the motion could be accommodated along the zone of a known structural and geological boundary. The proposed post-Late Cretaceous left lateral motion (~400 km) of East and West Antarctica is entirely speculative. The absence of geophysical data in the eastern Ross Sea precludes an evaluation of our hypothesis for this area, although we would expect to see evidence of limited subduction along the Marie Byrd Land continental margin if our hypothesis is correct.

The best test may come through investigations of the geology and tectonic evolution of other portions of the West Antarctic such as the Antarctic peninsula and adjacent seafloor, where the effects of our proposed plate motions must influence the geology there. It is also possible that the motion of East and West Antarctica we are proposing could be manifested as a rotation and extension²⁷ rather than pure strike-slip motion. If this were the case, the structural boundary through central Antarctica would represent an area of crustal extension and direct evidence for such an extension might be found by carefully re-examining aeromagnetic data across this area.

Tasman Sea Creation

The Central Tasman Sea was created by spreading about a now buried ridge that was active only from 80 to 60 m.y. BP. The buried axis and the pattern of magnetic lineations are offset along inferred fracture zones that are roughly orthogonal to the ridge and the lineations. Because of their similarity in heat flow, crustal properties and a strong suggestion of lineated magnetic anomalies, it seems likely that some other marginal

basins (such as the West Philippine Basin and South Fiji Basin) evolved with histories parallel to that of the Central Tasman Sea.

The Southern Tasman Sea began forming later, ~50 m.y. ago, in direct relationship to Australia's northward migration away from Antarctica through processes of seafloor spreading. The magnetic and morphologic properties of the central and southern regions of the Tasman Sea are nearly orthogonal and the fracture zone fabric of the former may have controlled the orientation of the ridge fabric of the latter.

A distinct basement and morphologic boundary separates the two Tasman Sea regions. To the north the total sediment blanket is thick and the near absence of internal reflectors indicates a restricted environment of hemipelagic sedimentation characteristic of an enclosed sea with limited sediment provenances and few oceanic circulation changes.

Continental reconstructions based on magnetic lineation data for the Tasman Sea and South-east Indian Ocean lead us to the conclusion that unrecognized post-Cretaceous plate motions must have occurred between Lord Howe Rise and the Campbell Plateau or between East and West Antarctica. These motions are conjectural, but we favour a hypothesis based on motion across a hypothetical Antarctic "sphenochasm" or tectonic discordance lying along the structural boundary between East and West Antarctica. Such motions would have important ramifications in unravelling the geologic and tectonic history of West Antarctica.

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LETTERS TO NATURE

PHYSICAL SCIENCES

Solar Neutrinos, Martian Rivers, and Praesepe

THE detection rate of solar neutrinos is significantly below the value predicted by contemporary theories of solar structure and evolution^{1,2}. The most likely—or at least best advertised—explanation of the discrepancy is episodic and probably periodic core expansion in the Sun³; in the models developed to date, mixing of ³He, produced by the p-p process, is the initiating event⁴⁻⁷. Although the radiative transfer time scale of photons from the core to the photosphere of the Sun is long, all the models identify epochs of anomalously low neutrino flux with epochs of anomalously low surface luminosity; and the temptation to connect the present “ice age” on Earth with low solar luminosity, L , has been unsuccessfully resisted. The peak-to-peak variations in L , $\Delta L/L$, range from about 7% to about 35% in these models, enough to have significant climatological consequences (see, for example, ref. 8). The duration of the variation is roughly the Kelvin-Helmholtz time scale $\sim 10^7$ yr; to correspond to the principal glaciation period, the interval between mixings must be $\sim 2.5 \times 10^8$ yr.

At present, conditions on the planet Mars are clearly those of an ice age climatology. A suggestion that much more clement conditions periodically recur on Mars⁹ has seemingly been confirmed by the widespread presence of sinuous dendritic channels, sharply concentrated towards the planet's equator (refs. 10, 11 and unpublished results of C. S., O. B. Toun and P. Gierash). Many of these channels can be understood only in terms of flowing surface liquid water. The higher pressures, temperatures, and water abundances required to excavate these channels point to more Earth-like epochs on Mars. A general discussion of climatic instability on Mars (C. S. *et al.*, unpublished results) identifies several possible mechanisms; one of them is $\Delta L/L$ variations of 10 to 20%, with the present epoch near the solar luminosity minimum. Because of the permanent presence of oceans on Earth, and the fact that no significant atmospheric constituent condenses at the surface of the Earth, it is possible that climatic excursions on Earth are more damped, for a given $\Delta L/L$, than on Mars.

Such luminosity variations, if they occur, are unlikely to be restricted to the Sun. Other evolved main sequence stars should experience similar fluctuations. The colour-magnitude diagrams of open and globular clusters might, in principle, be used to test for such $\Delta L/L$. Were the effects of ³He mixing to move a star exactly parallel to the main sequence, there would be no observable consequence of such $\Delta L/L$. But at least some of the published stirred models move stars at an angle to the zero age main sequence, and the width of the observed main sequence may therefore provide evidence on the existence and magnitude of such $\Delta L/L$.

Cluster colour-magnitude diagrams provide the obvious test because age and composition differences should not appreciably broaden the main sequence of an individual cluster. The cluster must not be too distant, otherwise the photometric errors will be excessive owing to faintness and crowding, and

For this reason all globular clusters, and such old open clusters as M67, NGC188 and NGC752, are excluded. On the other hand, the cluster must be sufficiently old to have produced ³He in the cores of its solar-type stars. Also, the cluster stars must be older than the time required to produce the first core-mixing cycle—possibly several $\times 10^8$ yr for stars near $1 M_{\odot}$. Finally, there must be an adequate number of stars not much more massive than $1 M_{\odot}$, because it is the p-p cycle which produces ³He rather than the C-N cycle (although C-N luminosity instabilities have not been excluded).

Open clusters such as the Pleiades are possibly near enough, but they are too young. Such clusters as the Hyades, Ursa Major, and Coma Berenices clusters may be sufficiently evolved, but they are too close—they subtend several degrees, and the apparent magnitude difference between the front and back of such clusters contributes significant scatter in the colour-magnitude diagram. (The Ursa Major cluster may be just close enough to separate out this effect through improved trigonometric parallaxes.)

The two remaining open clusters which may prove useful for our purpose are M39 and Praesepe. M39, however, has only about twenty stars and a visual extinction of 0.2 mag. Praesepe suffers none of these difficulties. At a distance of 159 pc it has no measurable reddening. It comprises ~ 100 stars, enough for meaningful statistics, and its turnoff age is $\sim 3 \times 10^8$ yr, probably old enough for our purpose.

The low luminosity end of the Praesepe colour-magnitude diagram has not evolved far enough to encounter a single mixing event. The stars at that end should be close to the zero age main sequence, and the scatter in the low luminosity main sequence should be representative of the observational errors. But more luminous stars, within a few magnitudes of the red giant turnoff, are sufficiently evolved to have experienced such mixing, if it occurs.

The photoelectric photometry of Praesepe was performed by Johnson¹², who, notably, remarked that the main sequence appeared distinctly wider for Praesepe stars earlier than about spectral type G0 than for later types. He quotes as an upper limit to the intrinsic scatter of the later type main sequence a probable error of ± 0.03 mag, corresponding to a standard deviation of ± 0.05 mag. From Johnson's data we find a standard deviation for the main sequence stars in Praesepe between $(B-V)=0.3$ and 0.6 of about ± 0.1 mag. Both estimates attempt to exclude double stars far above the main sequence. Thus, the square root of the differences between the upper and lower main sequence variances is nearly 0.1 mag in the correct sense: the more evolved stars show more scatter. (This estimate includes Johnson's quoted observational probable error of ± 0.022 mag.) With a dispersion of 0.1 mag in the evolved main sequence of Praesepe, the total excursion in luminosity of individual evolved stars is $\sim 15\%$. In fact the total $\Delta L/L$ of an individual star will be somewhat greater, because the excursions in the colour-luminosity diagram have a component parallel to the main sequence which is undetectable by this method. Thus the Praesepe colour-magnitude diagram implies that solar type stars experience luminosity excursions of just the order apparently required to resolve the solar neutrino and Martian river problems.

The colour-magnitude diagrams of other evolved clusters give dispersion of this order or larger, but the observational errors are also larger. Thus cluster photometry is consistent

with, but not necessarily uniquely indicative of, substantial variability in the solar luminosity on time scales $\sim 10^8$ yr. The fact that the Praesepe stars are approximately uniformly distributed through the width of the main sequence suggests that the excursion time off the main sequence is comparable with the time between mixings. Improved observation of Praesepe—and, possibly, M39 and the Ursa Major cluster—can shed further light on this problem. To the extent that climatic change caused by major solar variability produced major evolutionary advances on Earth, comparable biological evolution could have occurred on all other inhabited planets in the Galaxy.

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Effect of Low-level Radioactive Silver on Photographic Emulsions

LINDNER *et al.*¹ described the detection of low levels of radioactivity in silver bullion bars. Because this silver may be used in the manufacture of photographic emulsions the effects of the radioactivity are obviously of concern to photographic film manufacturers. We consider here the storage of photographic materials containing silver of the radioactivity level (10^{-4} $\mu\text{Ci/g}$ silver) given by Lindner *et al.*

We illustrate this point by calculating the rate of fogging that would result in a highly sensitive X-ray film for materials testing. Typically, such a film will be coated with about 2.3 mg of silver cm^{-2} . The silver bromide grains will have a diameter of about 1.5 μm . If such a film is irradiated with about 80 mR of γ -rays (0.3–2 MeV) an increase in optical density of about 0.3 will be the result. In a normal environment background, dose rate from cosmic radiation and radioactivity in the surroundings will be 80–100 mR yr^{-1} . Film manufacturers and users will rule out a film which, during storage, has obtained a fog level of optical density 0.3.

The film we describe will have about 3.5×10^8 grains cm^{-2} . With 10^{-4} $\mu\text{Ci g}^{-1}$ silver one will have a radioactive decay in 2.7×10^5 grains $\text{cm}^{-2} / \text{yr}^{-1}$. Thus about one per thousand of the grains will be directly involved in each year. If every radioactive decay involved one grain only, there would be no serious fogging, but the question "How many are involved?" is not a trivial one.

As a first step, the decay of $^{110\text{m}}\text{Ag}$ can be simplified. We will assume: for β s 0.087 MeV (127%), 0.529 MeV (26%), and

1.5 MeV (0.6%); for γ s 0.67 MeV (127%), 0.88 MeV (139%) and 1.4 MeV (38%). The 0.087 MeV β -rays will only involve one grain. The amount of silver involved with the 0.529 β s we will leave open.

To calculate the effect of the γ s we assume that the film is stacked with fifty sheets per cm thickness. If film and polyester film base were to be ground together, we would get a substance with a density of about 1.5 g cm^{-3} and with absorption coefficients μ_s of 0.105 cm^{-1} (0.67 MeV), 0.090 cm^{-1} (0.88 MeV), and 0.072 cm^{-1} (1.4 MeV). The radioactivity of this substance would be 0.43 d.p.s. per cm^3 , and result in a photon emission S_v of 0.56 $\text{s}^{-1} \text{cm}^{-3}$ (0.67 MeV), 0.61 $\text{s}^{-1} \text{cm}^{-3}$ (0.88 MeV) and 0.167 $\text{s}^{-1} \text{cm}^{-3}$ (1.4 MeV). The photon flux in the centre of an infinite slab source of homogeneous activity is

$$\phi = B S_v [1 - E_2(\mu_s d)] / \mu_s$$

where B is the build-up factor, $E_2(\mu_s d)$ is the exponential integral of the second order and the thickness of the slab is $2d$. If d is about 15 cm, $\mu_s d$ will be > 1 ; $E_2(\mu_s d) \sim 0.1$, so that $[1 - E_2(\mu_s d)]$ can be neglected. Neglecting the build-up factor for the time being we obtain

$$\phi \sim S_v / \mu_s$$

and with the data given we obtain photon fluxes of 5.3 $\text{s}^{-1} \text{cm}^{-2}$ (0.67 MeV); 6.8 $\text{s}^{-1} \text{cm}^{-2}$ (0.88 MeV); and 2.3 $\text{s}^{-1} \text{cm}^{-2}$ (1.4 MeV) to give dose rates of 7×10^{-6} R h^{-1} (0.67 MeV); 12×10^{-6} R h^{-1} (0.88 MeV); and 6×10^{-6} R h^{-1} (1.4 MeV). Adding these fluxes gives a dose rate of 210 mR yr^{-1} . This result is obtained without taking a build-up factor of, perhaps, 2–5 into consideration. Strong fogging in this case is evident.

Workers requiring very sensitive emulsions must hope that the case described by Lindner *et al.* will be an isolated incident. Or will we have to purchase low-level counting equipment for raw materials control?

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Radioactive Silver in East European Silver Bars

THE discovery of slightly radioactive silver in the form of the isotopes $^{108\text{m}}\text{Ag}$ and $^{110\text{m}}\text{Ag}$ in East European silver bars¹ is of considerable interest, because so far as is now known these short-lived radioactive isotopes have not been observed in natural materials². Lindner *et al.*¹ have given four possible explanations for the presence of the radioactive isotopes, one of which is that the silver ore was mined by an underground nuclear explosion. An alternative natural explanation for the phenomenon seems more probable.

Some of the silver deposits in Eastern Europe contain uranium, chiefly as pitchblende. Most of these are veins and not particularly amenable to nuclear mining. Those in the Bohemian massif (Jáchymov) are well known and there are others in the Příbram area of Czechoslovakia and in the Tertiary and older rocks in the great metalliferous belt that extends through a number of Eastern European countries to the Black Sea.

The production of the $^{108\text{m}}\text{Ag}$ and $^{110\text{m}}\text{Ag}$ isotopes is essentially caused by slow neutron bombardment, the neutron capture reaction involving ^{109}Ag and ^{110}Ag being



In uraniferous silver deposits there are several neutron sources. Some of these are the various (α, n) reactions involving alpha particles from uranium and its decay products and various light elements such as Li, Be, B, and F, all of which occur often in some quantity in uraniferous silver deposits. There is also a source of neutrons in the spontaneous fission of uranium. According to Segrè³ and Littler⁴ the number of neutrons spontaneously emitted from natural uranium is $1.5\text{--}1.65 \times 10^{-2} \text{ g}^{-1} \text{ s}^{-1}$. This is a relatively low neutron density, but probably sufficient to produce low levels of $^{108\text{m}}\text{Ag}$ and $^{110\text{m}}\text{Ag}$ in silver ores. When one considers the radioactive equilibrium conditions, the intimate contact of silver and uranium minerals in certain uraniferous silver deposits and the concentration factor ($>5,000$) inherent in winning the silver from the ore, it seems probable that both $^{108\text{m}}\text{Ag}$ and $^{110\text{m}}\text{Ag}$ would be concentrated in the silver bars in measurable amounts.

To test this speculation, I suggest that a search be made for small concentrations of $^{108\text{m}}\text{Ag}$ and $^{110\text{m}}\text{Ag}$ in silver minerals from uraniferous silver deposits (Great Bear Lake, Canada; Jáchymov, Czechoslovakia) by well equipped laboratories using appropriate detection apparatus.

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Near-bottom Thermocline in the Samoan Passage, West Equatorial Pacific

ANTARCTIC bottom water (ABW) flows into the Pacific basin from the region between Macquarie Island and Antarctica¹⁻³. Dynamic considerations suggest that the bottom water flows northwards as an intense current along the western boundary of the Pacific basin^{4,5}. Oceanographers have noted that the Pacific is divided into at least four principal basins which are separated by relatively shallow sills that constrain the flow of water below 4,000 m (refs. 3, 6, 7). The narrow Samoan Passage, first recognized by Reid⁸, is perhaps the most important channel with depth sufficient to allow a significant flow of bottom water from the southern basin into the central basin. Bottom current velocities in the passage of 5 to 15 cm s⁻¹ towards the north have been

measured⁹ and Nansen casts in the passage revealed a sharp decrease in temperature of 0.27° C in 150 m at a depth of about 4,400 m¹⁰. This benthic thermocline marks the abrupt boundary between the deep water and the ABW and supports the observation of cold bottom water flow through the passage. (In a personal communication, P. Lonsdale and J. Reid suggested that the bottom water may include entrained high salinity Atlantic deep water as well as ABW.)

In the autumn of 1971, thirteen temperature profiles were taken with a thermal gradient recorder mounted on a sediment corer in and near the Samoan Passage in order to define the flow of bottom water through the area. The resistance of a precisely calibrated thermistor attached to the top of the instrument was recorded every 30 s as the corer was lowered to and raised from the bottom. A pinger was mounted 78 m above the core weight so that a time record of the depth of the instrument could be recorded aboard ship. The temperature time data recorded on film in the heat flow instrument were then merged with the depth/time data to yield *in situ* water temperature profiles. The relative precision of the thermistor was 0.002° C and the absolute accuracy about 0.01° C. The error in depth determination was ~20 m near the bottom but could be as much as 150 m at a distance of 1.5 km above the bottom because of rapid changes in bathymetry and variations in the rate of film speed in the thermal gradient recorder. This estimate of the error in the depth determination is in agreement with the magnitude of discrepancies in the temperature profiles obtained from the lowering and subsequent raising of the instrument at the same station.

A pronounced benthic thermocline was present in all eight of the new temperature profiles taken in the axis of the passage (Figs. 1 and 2). A temperature decrease of 0.31° C (1.37° C to 1.06° C) occurs over a depth interval of about 250 m at a depth of 4,200 to 4,400 m. Below 4,400 m there is a slight increase in temperature because of adiabatic compression. The thickness of the ABW using the 1.10° C isotherm (*in situ*) is 800–950 m in the axis of the passage (Fig. 2). The thickness of the layer decreases near the walls of the passage in such a way that the boundary between the deep water and the bottom water (about 1.10° C) remains at a depth of about 4,200–4,400 m. At the northern end of the passage, the depth of the benthic thermocline increases while the thickness of the bottom water layer decreases (Fig. 2). The benthic thermocline is not clearly present in four of the five temperature profiles taken north of the passage. The profiles do show a slight decrease in temperature (0.05° C, see northernmost temperature profile in Fig. 1) and a warmer isothermal layer. The sharp boundary between the bottom and deep water may be obscured by turbulence as the water flows over the sill to the north (P. Lonsdale, personal communication).

The temperature data suggest that the wedge of bottom water flowing northwards is narrowest and thickest in the axis of the passage (Fig. 2). During Chain 100, two current meters were placed in the axis of the passage (8° 15' S,

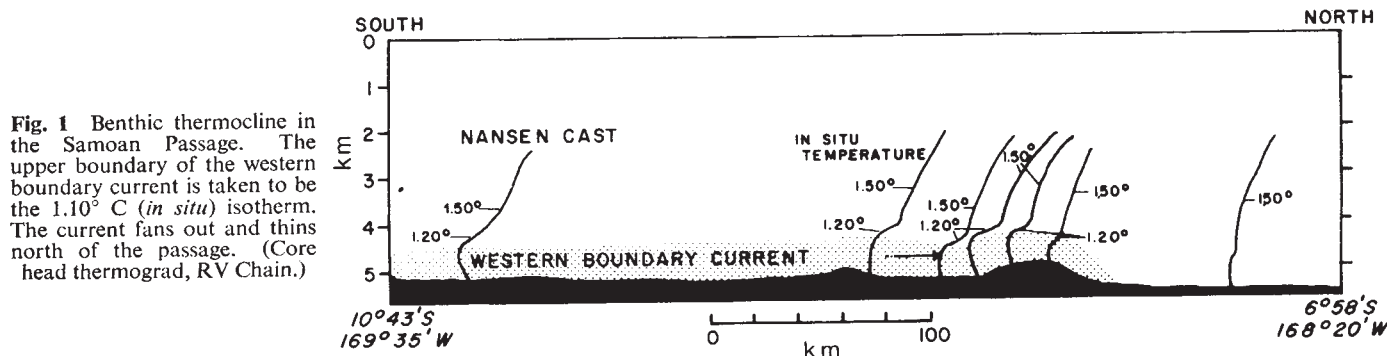


Fig. 1 Benthic thermocline in the Samoan Passage. The upper boundary of the western boundary current is taken to be the 1.10° C (*in situ*) isotherm. The current fans out and thins north of the passage. (Core head thermograd, RV Chain.)

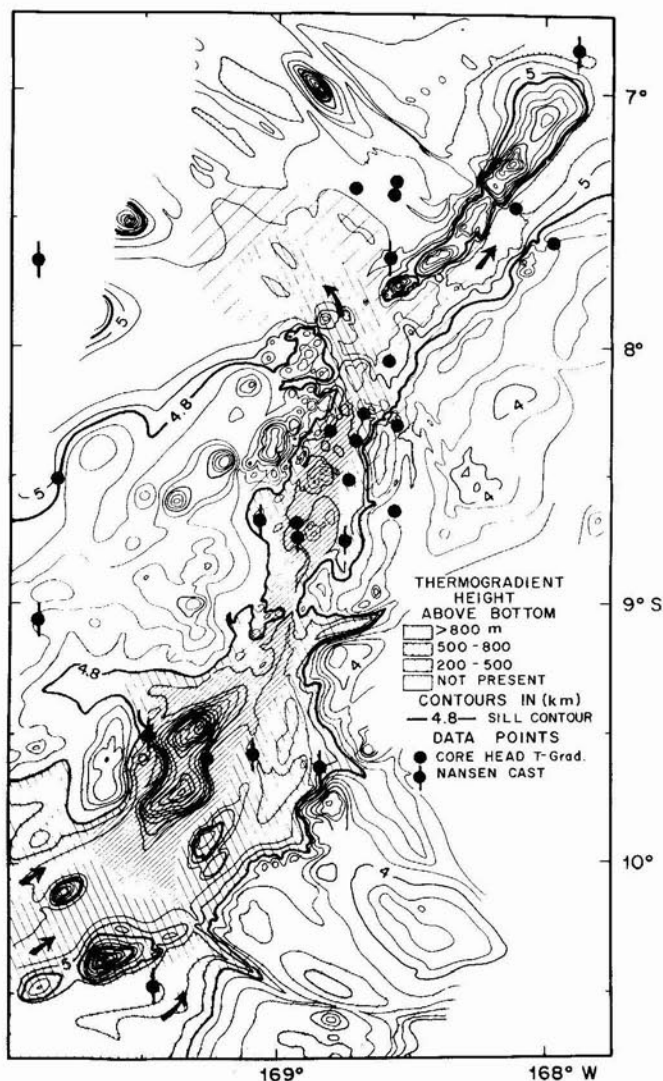


Fig. 2 Isothermal layer thickness in Antarctic bottom water. The bottom water layer is thickest in the narrow axial zone of the passage. North of the passage the flow bifurcates, with most of the water flowing to the north-west. The bathymetry was contoured by P. Lonsdale based on data from the Chain 100 and Styx expeditions. Recently acquired data indicate that the north-west channel may be narrower than shown.

168° 43' W, and 8° 16' S, 168° 40' W) for 139 h and 25 h, respectively, at a distance of 3 m from the bottom. A mean velocity of 9.3 cm s^{-1} in a northerly direction was recorded, with a maximum velocity of 16 cm s^{-1} . Using a topographic cross-section at 8° 30' S, and an average velocity of 9.3 cm s^{-1} , we derive a transport of about 3 Sverdrup ($10^6 \text{ m}^3 \text{ s}^{-1}$) of cold bottom water flowing from the southern basin into the central basin of the Pacific.

North of the passage, a topographic high creates two channels, one striking north-east, the other north-west. Temperature data show a 320 m thick layer of bottom water in the axis of the north-easterly passage. A current meter placed near the east side of the channel for 3 d recorded a mean velocity of 2 cm s^{-1} with a maximum of 4.9 cm s^{-1} . But the direction of flow was north-east for 1.5 d and south-west for 1.5 d, so the net flow is near zero. A more constant northerly flow may exist in the axis of the north-east channel. The lack of a sharp benthic thermocline in the four temperature profiles near the topographic high north of the passage, and the low flow through the north-easterly channel, suggest that most of the flow (about 3 Sverdrup) is directed towards the north-west. The greater

ion due to Coriolis forces (although weak at this latitude) would favour a greater flow to the north-west.

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Changes in the Isotopic Composition of East Mediterranean Seawater during the Holocene

WHEN molluscs secrete their shells, the $\delta^{18}\text{O}$ in the shell is a function only of the temperature and of the $\delta^{18}\text{O}$ in the water¹⁻⁴. Thus one can compute the temperature at which an ancient shell was formed by measuring its $\delta^{18}\text{O}$ value if one assumes that of the water in which it grew. For Pleistocene samples there has been a heated discussion as to the degree of change in the $\delta^{18}\text{O}$ of the sea⁵⁻⁷.

The present Eastern Mediterranean (EM) is more saline⁸ and has a higher $\delta^{18}\text{O}$ than the Western Mediterranean (WM). Here we consider whether this difference always existed and, if not, when it occurred. We measured the oxygen isotopic composition and ^{14}C ages of samples of the aragonite forming *Glycymeris violaceus* from several locations (present and fossil shorelines) along the Israeli coast (Fig. 1).

The geological setting of these samples will be described elsewhere. Each shell specimen was cleaned by washing and by boiling in H_2O_2 . This was followed by grinding and X-ray diffraction analysis to ascertain that no recrystallization to calcite had occurred. Carbon dioxide was extracted by the method of McCrea⁹ and duplicates were run in a double collecting Atlas M-86 mass spectrometer.

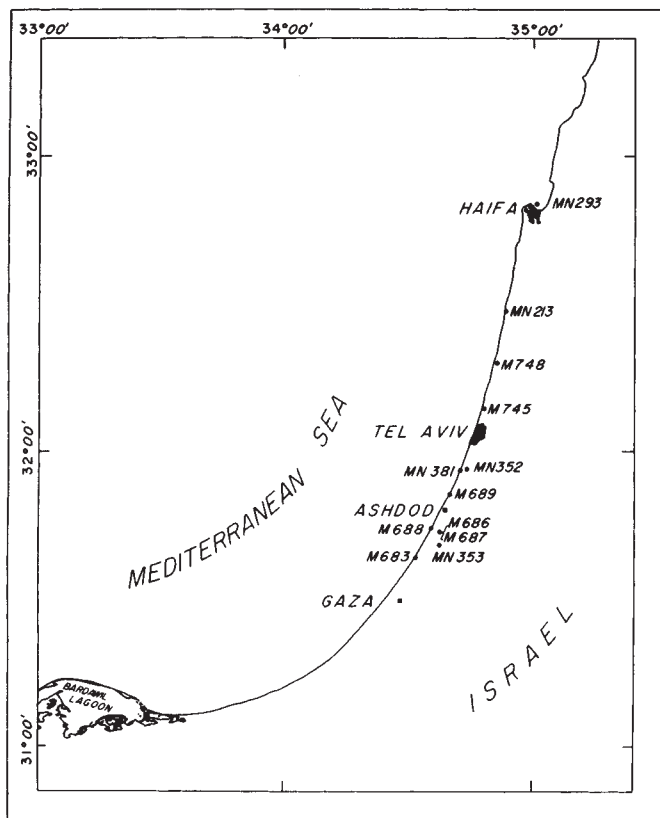


Fig. 1 Map of the coast of Israel showing locations where *Glycimeris* shells were collected for ^{18}O and ^{14}C analyses.

Carbon-14 analyses were performed as described by Carmi *et al.*¹⁰. Because such an analysis requires up to thirteen specimens and ^{18}O analyses are performed on single specimens, we use the term "sample" to refer to the entire group analysed from any location, although ^{18}O was determined for every specimen in the sample.

Table 1 shows the location, number of specimens analysed in each sample, ^{14}C age, average $\delta^{18}\text{O}$ and standard deviation in $\delta^{18}\text{O}$ for the specimens of each sample. In nearly half of the cases this standard deviation is equal to the analytical error. We encountered one sample (from a submarine drill core off Nahal Alexander) which had so large a deviation (0.93) that it was obviously a mixture of populations. For this reason no ^{14}C analysis was performed on it.

Because the present $\delta^{18}\text{O}$ of the EM is +0.18‰ (SMOW), from the equations of refs 3 and 4, we calculate the temperature for sample MN293 to be 25°C. This is equal to the average spring to autumn temperature for Israel coastal waters¹¹. (Molluscs generally secrete their shells in this period.)

Table 1 Ages and $\delta^{18}\text{O}$ Values of the Samples Analysed

Sample No.	Location	No. of specimen	Age (yr)	$\delta^{18}\text{O}$ ‰	
				Average	Standard deviation
MN293	Haifa	3	0	-0.014	0.005
MN394	Principality of Monaco	3	0	-0.16	0.020
MN381	Palmachim	9	1,150	0.00	0.053
M688	Ashdod	9	1,500	0.016	0.028
M686	Ashdod Yam	10	2,120	0.018	0.037
M687	Ashdod Yam	13	2,360	0.041	0.024
M683	Ashkelon	6	2,610	0.033	0.010
M745	Tel Baruch	6	2,920	0.015	0.030
MN213	Caesaria	8	3,300	-0.050	0.014
M689	Rogozin	5	3,800	0.04	0.020
M748	Nahal Poleg	8	3,800	-0.070	0.020
MN352	Nahal Soreq	8	4,600	-0.078	0.040
MN353	Nitzanim	6	4,900	-0.147	0.045

In Fig. 2a we show an overall spread of 0.19‰ in the $\delta^{18}\text{O}$ of the population averages. According to the Craig equation³ such a spread must be due to variations in the temperature and/or the $\delta^{18}\text{O}$ of the water. Thus it can be explained in one of the three following ways:

(1) By assuming that $\delta^{18}\text{O}$ in the water was always what it is today. Fig. 2b shows the temperatures calculated from this assumption. We conclude that this assumption is very improbable because of the large temperature range (9°C) over this time interval. Other evidence suggested that the temperature range for this interval is no more than 2°C (ref. 12).

(2) By assuming that the water temperature has not changed. Fig. 2c shows the $\delta^{18}\text{O}$ of the water as calculated from this assumption. But Emiliani *et al.*¹ show that temperature variations have occurred in the WM.

(3) By assuming that water temperatures in the EM have varied in the same way as in the WM¹ but that there was always a 4°C difference between EM and WM (which is the difference today). Thus neither temperature nor $\delta^{18}\text{O}$ of the water remained constant. Figure 2c shows the $\delta^{18}\text{O}$ of the water calculated on this assumption.

We have shown that the first two hypotheses are improbable so we are inclined to accept the third one. The consequences are that the $\delta^{18}\text{O}$ remained approximately at the present value for the past 2,800 yr, and that between 2,800 and 4,900 yr BP there was a change in $\delta^{18}\text{O}$ of the EM water from +0.2 to +0.1‰ (SMOW). We note that the value of $\delta^{18}\text{O}$ calculated for EM water 4,900 yr BP is similar to that of present WM (0.1‰).

How can this change have arisen? It is well known that in large seas high $\delta^{18}\text{O}$ values are accompanied by high salinities. This is explained as the consequence of increases in both parameters as a result of an excess of evaporation over precipitation. At present there is a net excess of evaporation over precipitation of 0.8 m.y.⁻¹. Thus, our data imply that no such excess existed in the EM 4,900 yr BP.

We do not know what may have caused so sharp a change

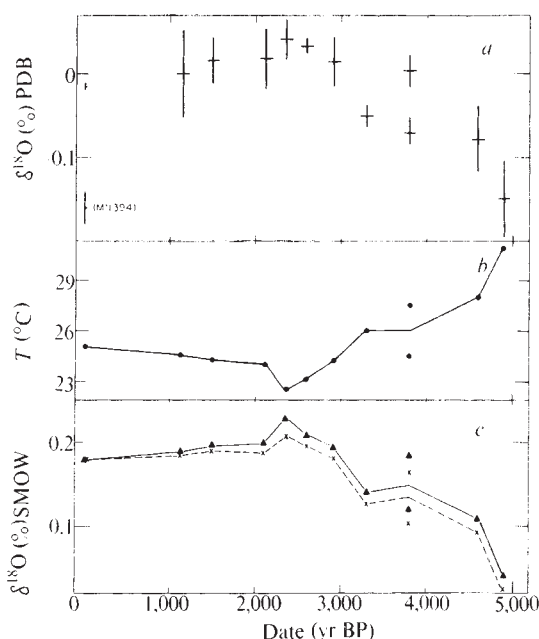


Fig. 2 a, Observed ^{18}O as a function of age for the *Glycimeris* samples from Israel. The point labelled MN394 is from Monaco and is shown for purposes of comparison. b, Calculated temperatures as a function of age for the samples shown in Fig. 2a based on assumption (1). c, Calculated water ^{18}O as a function of age for the samples shown in Fig. 2a. Δ , Based on assumption (2); $\times$, based on assumption (3).

in the evaporation-precipitation balance in the EM 4,900 yr BP. It seems that the small temperature change detectable would be insufficient to explain this. One possibility is a change in wind direction. Another possibility is a tectonic change which caused a rise in the sill of the Sicilian strait and impaired the contact between the EM and the WM. Supporting evidence for a change from humid to dry climate at about 5,000 yr ago is found in palynological studies of the Northern Mediterranean region¹³ and of Northern Israel¹⁴.

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Recent Rainfall Trends in Africa, the Middle East and India

IN the Middle East and Africa north of the Sahara the wet season extends from October to May; small amounts of rain also fall in Pakistan and north-west India at that time of year. Winter-spring rainfall in these areas is controlled primarily by divergence patterns in pressure troughs in the mid- and upper-tropospheric circumpolar westerlies. Rainfall is associated with the widespread ascent of warm, moist air from the tropics on the eastern side of the troughs, and also with deep convective instability in the cold air at, and slightly to the rear of, the trough axes, especially over coasts and mountains. It is in winter that the increase in the latitudinal temperature gradient in the northern hemisphere causes the westerly circulation to increase in strength and the westerly troughs to penetrate into sub-tropical latitudes. In summer, the latitudinal temperature gradient is at a minimum, the general circulation is relatively weak, and the westerly troughs are of small amplitude and restricted to middle and high latitudes.

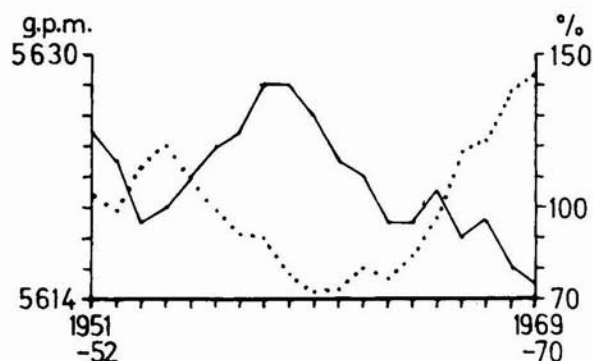


Fig. 1 , Five-year running means of the mean percentage of normal seasonal rainfall (October to May) at Bikaner (India), Shiraz (Iran), Mosul (Iraq), Beirut (Lebanon), Jerusalem (Israel), Jiddah and Riyadh (Saudi Arabia), Nalut (Libya), Marrakech (Morocco) and Atar (Mauritania); —, five-year running means of the mean altitude of the 500 mbar surface (geopotential metres) along 40° N, between 110° W and 70° E, from October to May (data extracted from *Die Grosswetterlagen Europas* of the German Weather Service (1949–1972)).

In order to study recent rainfall trends over North Africa, the Middle East and north-west India, seasonal totals from ten widely separated stations (Fig. 1) were used, with mean values ranging from 24 to 893 mm. Figure 2 shows that the rainfall trends, although somewhat out of phase, are similar at widely separated stations with vastly different rainfall totals. The seasonal rainfall at each station was expressed as a percentage of the normal, and the mean percentage of all the stations determined, thus giving a crude area-mean seasonal rainfall figure. Smoothing was achieved by computing five-season running mean values (Fig. 1). The trend from the mid-1950s to the early 1960s was towards lower seasonal rainfall, but the trend has since been completely reversed.

Winter-spring rainfall in these sub-tropical regions is particularly sensitive to the southward extent of the troughs in the mid-tropospheric circumpolar westerlies. Thus, the altitude of the 500 mbar surface along 40° N is a meaningful index of the southward extent of the westerly troughs for analysing rainfall deviations (my unpublished work). The five-season running means of the mean altitude of the 500 mbar surface along 40° N, between 110° W and 70° E, from October to May are shown in Fig. 1.

Figure 1 shows that, since 1951/52, the altitude of the 500 mbar surface along 40° N and the winter-spring rainfall over north Africa, the Middle East and north-west India have been highly negatively correlated: the correlation coefficient is $r = -0.74$. The decrease in the altitude of the 500 mbar surface during the 1960s signifies an increase in the southward extent of the troughs in the circumpolar westerlies. In the late 1960s the seasonal rainfall totals over large areas of north Africa and the Middle East were the highest ever recorded.

This tendency, towards northerly meridionality in the general circulation of the northern hemisphere, has also been reflected in an excessive amount of sea ice in the European sub-Arctic, and a decline in the frequency of westerly winds over Britain¹. There are also repercussions in the summer monsoon rainfall areas to the south of the Sahara and in south-west Asia, where

Table 1 Five-year Running Mean Percentage of Normal Seasonal Rainfall Centred on 1957 and 1970

Country	India	India	Sudan	Niger	Mali	Mali	Mauritania	Mauritania	
Station	Bikaner	Jodhpur	Khartoum	Agades	Tessalit	Gao	Nouakchott	Atar	Mean
1957	114	115	122	130	140	114	106	121	120
1970	71	68	80	44	63	75	74	52	66

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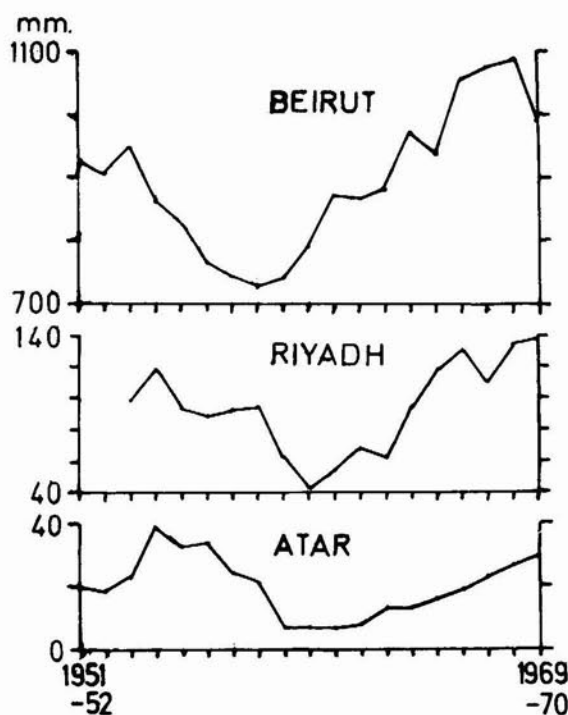


Fig. 2 Five-year running means of seasonal rainfall (mm) at Beirut (Lebanon), Riyadh (Saudi Arabia) and Atar (Mauritania).

the synoptic scale systems controlling rainfall are quite different from those mentioned above. From Mauritania across to north-west India the summer monsoon rainfall has decreased steadily by more than 50% since 1957; Table 1 shows the five-year running mean percentages of normal seasonal rainfall centred on 1957 and 1970 for eight stations towards the northern limit of the monsoon rains. These are the areas where rainfall is marginal for agriculture and where the failure of the monsoon rains can be economically disastrous. There have been press reports recently of widespread and severe drought in western India, Chad, Niger, Upper Volta, Dahomey, Mali, Senegal and Mauritania. It is not unusual for the monsoon rains to fail in some of these regions each year, but it is almost unknown for them to fail so widely in several consecutive years. It is probable that the abnormal southward extent of the troughs in the circumpolar westerlies has restricted the northward extent and intensity of the tropical circulation systems controlling the monsoon rainfall, and also the mean meridional circulation of the Hadley Cell. A similar relationship between large-scale eddies in the circumpolar westerlies and the vertical motions in the Hadley circulation has been found in laboratory experiments²: with short waves in relatively weak circumpolar westerlies, a meridional circulation was evident in the tropics; but as the circumpolar westerlies expanded the tropical meridional circulation broke down.

The results provide further evidence of recent changes in the general circulation of the northern hemisphere and the equatorward shift of climatic zones; a trend which was first evident in the 1930s (ref. 3), but which became even more marked in the 1960s.

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Catecholamines in the Median Eminence: New Evidence for a Major Noradrenergic Input

THE median eminence of the hypothalamus contains large amounts of hypothalamic hormones (releasing factors)¹, but the cellular localization of these hormones is unknown. In contrast, there is convincing evidence, from fluorescence histochemical studies, for the existence of a dopaminergic system with neuronal cell bodies in the arcuate nucleus, whose axons project into the external layer of the median eminence². Deafferentation studies combined with fluorescence^{3,4} or electron microscopy⁵ have suggested that there may also be a noradrenergic component in the innervation of the median eminence. We now report additional biochemical evidence supporting the existence of a substantial noradrenergic input to this brain area. We also describe the properties of a dopamine (DA) uptake system in the median eminence.

Wistar male rats weighing 150–250 g were used. The median eminence was removed using a stereomicroscope. The pia mater was peeled off and the median eminence removed by dissecting the lateral and frontal borders (defined by the presence of capillary loops) while holding the stalk with fine forceps; the stalk was then removed. The average weight of such samples was approximately 0.3 mg. To excise the arcuate nucleus two coronal cuts were made in the remaining hypothalamus, the frontal cut at the level at which the optic tracts enter the brain, and the second at the pre-mammillary area. From these sections a triangular shaped tissue sample about 1 mm wide and 1 mm high was obtained from the medial and basal hypothalamus. The average weight of such samples was 1.5 mg.

The catecholamine content of the tissue was determined in pools of four to ten median eminences or arcuate nuclei homogenized in 15–20 vol of 0.1 M perchloric acid. Noradrenaline (NA) and DA were assayed in duplicate aliquots of the homogenate by sensitive and specific radiochemical enzymatic techniques. The technique for NA was a modification of that described by Iversen and Jarrott⁶ which exploits the phenylethanolamine N-methyl transferase (PNMT) catalysed methylation of tissue NA to adrenaline in the presence of ³H-methyl-S-adenosyl methionine. Dopamine estimation involved catechol-O-methyl transferase (COMT) catalysed methylation of this amine to tritiated 3-methoxytyramine (unpublished results of A. C. C. and L. L. I.). In each assay the labelled products were separated by paper chromatography, eluted and the radioactivity counted. The amounts of catecholamines in tissue extracts were determined by comparison with NA and DA standards. Using the PNMT method we measured as little as 1 ng of NA, and with the COMT method, as little as 2 ng of DA. Confirming the results from fluorescence microscopy², the concentration of DA in the median eminence was found to be very high (Table 1), comparable with that measured by this method or previously reported by fluorimetric assay procedures for the corpus striatum⁷. The DA content of the arcuate nucleus tissue, however, was below the sensitivity of the method (0.5 $\mu\text{g g}^{-1}$).

The results obtained with the PNMT method for NA in median eminence were unexpectedly high (Table 1). The individual values ranged from 2.7 to 5.4 $\mu\text{g g}^{-1}$ tissue, concentrations higher than previously reported in any other brain region. The concentrations of NA and DA, when assayed in parallel aliquots from the same tissue extracts, were similar. The possibility that the high NA values in the median eminence might have arisen through *in vitro* conversion of DA to NA, and thence to adrenaline in the PNMT

reaction is considered unlikely because NA estimations in the DA-rich corpus striatum with the same method failed to give values above the blank level.

A specific uptake mechanism for DA exists in the DA terminals of the corpus striatum⁸, and it was thus of interest to determine whether a similar mechanism exists in the median eminence. The fluorescence histochemical studies of other workers^{9,10} have suggested that catecholamine uptake does occur in terminals in the tubero-infundibular region. A modification of the method of Horn and Snyder¹¹ was used to study the uptake process in homogenates of pooled median eminence and arcuate nucleus tissue. Uptake of catecholamines into brain homogenates occurs predominantly into synaptosomes¹². Synaptosomes form when median eminence tissue is homogenized, and a recent study¹³ has demonstrated the *in vitro* release of amino-acids and releasing factors from such preparations.

Table 1 Noradrenaline and Dopamine Content of the Rat Median Eminence and Arcuate Nucleus

Tissue	Catecholamine	Concentration
Median eminence	Dopamine	10.3 ± 0.98 µg g ⁻¹ (5)
Median eminence	Noradrenaline	4.1 ± 0.53 µg g ⁻¹ (4)
Arcuate nucleus	Noradrenaline	1.3 ± 0.12 µg g ⁻¹ (3)

The catecholamine contents of pooled median eminence and arcuate nucleus tissue were estimated by radiochemical enzymatic methods (see text). Results are expressed as the mean value ± s.e.m. with the number of experiments in parentheses.

Tissue was weighed and then homogenized in 50 vols of 0.25 M sucrose and samples of homogenate equivalent to 0.2–0.4 mg of original tissue weight were incubated at 37° C in a Krebs–Henseleit medium containing small amounts of ascorbic acid, EDTA and a monoamine oxidase inhibitor (nialamide) to inhibit the spontaneous or enzymatic breakdown of labelled catecholamine. ³H-Dopamine (2-³H, SA=3.48 Ci mmol⁻¹; NEN Chemicals GmbH, Dreieichenhain, Germany) was present at a concentration of 1 × 10⁻⁷ M.

There was a rapid uptake of radioactivity giving an estimated tissue: medium ratio of up to 30:1 for the median eminence after incubation for 5 min at 37° C. The tissue: medium ratio is defined as d.p.m. g⁻¹ of original tissue weight per d.p.m. ml⁻¹ of incubation medium. The uptake process for ³H-DA in the median eminence had the properties of an active transport, similar to those of the catecholamines in other brain areas^{14,15}—it was temperature and sodium dependent (Table 2).

To explore the relative contributions of noradrenergic and dopaminergic neurones to this uptake we used drugs that have a selective inhibitory effect on the uptake of catecholamines into these two categories of nerve terminals. Desipramine, in particular, is about 1,000 times more potent as an inhibitor of the catecholamine uptake sites in noradrenergic nerve endings than in dopaminergic terminals^{16,17}. This drug was used at a concentration of 5 × 10⁻⁷ M, which from previous experience would cause about 90% inhibition of NA uptake sites, while leaving DA uptake unaffected¹⁶. In

Table 3 Effect of Desipramine on ³H-Dopamine Uptake

Tissue	³ H-dopamine uptake	
	Control	DMI-% control
Median eminence	100.0 ± 2.0 (4)	68.3 ± 3.1 (4)
Arcuate	100.0 ± 1.6 (3)	34.0 ± 1.2 (3)
Pituitary stalk	100.0 ± 6.5 (3)	63.0 ± 8.4 (3)
Striatum	100.0 ± 0.1 (6)	108.0 ± 0.1 (8)

The effect of desipramine (5 × 10⁻⁷ M) on the uptake of ³H-dopamine (1 × 10⁻⁷ M) by homogenates of median eminence, arcuate nucleus, pituitary stalk and striatum. Results are expressed in % of control as the mean value ± s.e.m. with the number of experiments in parentheses. Mean values for ³H-dopamine uptake in control experiments, expressed as tissue: medium ratios, were: median eminence=30; arcuate=29; pituitary stalk=92; and striatum=109.

contrast, benztropine is more selective in inhibiting catecholamine uptake into dopaminergic nerve endings¹⁶.

In the arcuate-median eminence complex desipramine (5 × 10⁻⁷ M) produced a 36% inhibition of ³H-DA uptake. When the arcuate nucleus, median eminence and hypophysial stalk were investigated separately desipramine was found to produce a more marked inhibition of ³H-DA uptake in the arcuate nucleus than in the other two areas (Table 3). At this concentration it caused no significant inhibition of ³H-DA uptake in the corpus striatum (Table 3). These results thus support the concept of a substantial noradrenergic input to the median eminence.

To investigate the properties of the uptake sites in dopaminergic neurones in the median eminence, without the complications of transport of ³H-DA into noradrenergic terminals, the effect of various concentrations of benztropine on ³H-DA uptake was investigated in the presence of 5 × 10⁻⁷ M desipramine. In such conditions, benztropine produced a dose-dependent inhibition of ³H-DA uptake into the median eminence homogenates (Table 4). The IC₅₀ value for benztropine of (9.0 × 10⁻⁸ M) was very similar to that previously obtained by Coyle and Snyder¹⁸ for inhibition of DA uptake in homogenates of the rat corpus striatum.

Table 4 Inhibition of Desipramine-insensitive ³H-Dopamine Uptake into Median Eminence Homogenates by Benztropine

Concentration of benztropine (M)	% Inhibition of H-dopamine uptake
1 × 10 ⁻⁶	75.5 ± 2.10 (4)
1 × 10 ⁻⁷	63.3 ± 6.74 (3)
5 × 10 ⁻⁸	32.0 ± 3.51 (4)
IC ₅₀ = (9.0 × 10 ⁻⁸ M)	

Benztropine at three concentrations was preincubated for 5 min at 37° C with median eminence homogenates in the presence of desipramine (5 × 10⁻⁷ M). ³H-Dopamine (1 × 10⁻⁷ M) was then added and the incubation continued for a further 5 min. Values for inhibition are the mean ± s.e.m. with the number of experiments in parentheses. The IC₅₀ is defined as the concentration of drug producing a 50% inhibition in substrate uptake. It was determined by a graphical method using log-probit paper¹⁶.

Our results give more precise quantitative information on the catecholamine content of the median eminence of the rat than has previously been available, and these data indicate that in this area of the brain the ratio of DA to NA is close to 2:1. The values reported here for DA concentration in the median eminence are considerably higher (with one exception¹⁹) than those previously published^{20–22}. This discrepancy may be because the present sensitive assay techniques allowed catecholamine estimations to be performed in relatively small, precisely dissected areas of hypothalamus. The less sensitive fluorimetric techniques previously used necessitate the pooling of large quantities (up to 100 median

Table 2 Effect of Sodium Removal and Low Temperature on Dopamine Uptake by Median Eminence Homogenates

Temperature	Medium	³ H-dopamine uptake as % control
37° C	Na ⁺ (control)	100 ± 4.3 (4)
37° C	Na ⁺ free	37.5 ± 3.1 (4)
0° C	Na ⁺	10.5 ± 2.9 (4)

Effect of sodium-free medium (NaCl replaced by 0.25 M sucrose) and low temperature on the uptake of ³H-dopamine (1 × 10⁻⁷ M) in Tris-Krebs medium (NaHCO₃ replaced by 5 mM Tris/HCl, pH 7.4) by median eminence homogenates. Results are expressed as the mean values ± s.e.m. with the number of experiments in parentheses.

eminences per sample) of material which may have been contaminated by neighbouring structures of low catecholamine content.

The report from fluorescence histochemical studies, that catecholamines are taken up in the arcuate-median eminence complex^{9,10}, has been confirmed and additional quantitative biochemical data are provided. In addition we have demonstrated that there is a substantial desipramine-sensitive component in such uptake, which was not detected in the previous studies¹⁰. The demonstration of a benztropine-sensitive uptake mechanism for DA in tubero-infundibular neurones also supports the view that the uptake system in these dopaminergic neurones has properties similar to those found in other neurones in the CNS. The catecholamine concentrations in the median eminence, together with the uptake results, indicate that the noradrenergic innervation of this structure is considerably larger than was previously suspected. Both DA and NA have been implicated in the neuronal regulation of adenohipophysial secretion²³, but more information is needed for an understanding of their precise physiological roles in the median eminence.

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Amino-acid Substitution in the $\alpha^1\beta^1$ Intersubunit Contact of Haemoglobin-Camden $\beta 131$ (H9) Gln $\rightarrow$ Glu

THE structures of more than 100 variant human haemoglobins have been determined and in many cases it has been possible to relate the position of the individual amino-acid substitution to the stability and function of the molecule^{1,2}. Here we describe the amino-acid replacement, structural alterations and oxygenation properties of a variant, haemoglobin-Camden, which has a substitution in the $\alpha^1\beta^1$ intersubunit contact of the haemoglobin molecule.

The variant was discovered during routine starch gel electrophoresis of red cell haemolysates from antenatal patients of African extraction, to exclude the presence of sickle cell trait. Investigation of the patient showed no anaemia or evidence of haemolysis: haemoglobin, 11.1 g per 100 ml. of blood; packed cell volume 41%; red cells, 4.4×10^9 ml.⁻¹; mean cell volume 93 μ m³; MCHC, 27G per 100 ml.; reticulocytes, 0.1%. There was no splenomegaly. Oxygen dissociation studies on the patient's red cells suspended in isotonic phosphate buffer at 37°C (ref. 3) gave values that were within the normal range: $P_{50} = 31.5$ mm Hg $n = 2.7$ at pH 7.13; $P_{50} = 22.0$ at pH 7.46; $P_{50} = 44.0$ at pH 6.47; Bohr shift (pH 7.46–7.13) = –0.47.

On starch gel electrophoresis in Tris-EDTA-borate buffer, pH 8.6, the variant haemoglobin-Camden migrated just ahead but was poorly resolved from the normal Hb-A, whereas in phosphate buffer at pH 6.9 no separation of the two haemoglobins was observed. These results excluded the possibility that Hb-Camden was Hb-J, as the latter is clearly resolved from Hb-A in both these systems. The proportions of the haemoglobins in the patient's haemolysates determined by densitometry in the pH 8.6 gel system were Hb-Camden 45% and Hb-A₂ 3.2%; the remainder was Hb-A. Complete separation of Hb-Camden and Hb-A was subsequently achieved by agar gel electrophoresis in citrate buffer, pH 6.2, the Hb-Camden migrating anodally to Hb-A.

As it proved difficult to isolate the variant haemoglobin by starch block electrophoresis or column chromatography, globin was prepared from the unseparated Hb-Camden and Hb-A. Chromatography of the globin on CM cellulose⁴ showed an abnormal chain eluting just before the normal β^A chain, the position of elution suggesting a single negative charge change from the β^A chain. Tryptic peptide mapping of the aminoethylated⁵ abnormal chain showed that β T13 was replaced by a more anodal peptide, the position of the new peptide corresponding to an extra negative charge on a peptide of that size (Fig. 1). After elution from the fingerprint, amino-acid analysis of this peptide showed the same

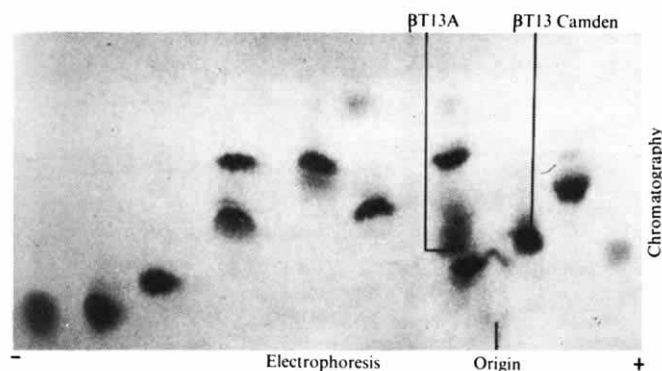


Fig. 1 Peptide map of the tryptic digest of the isolated β -chains of Hb-Camden. The normal β T13 peptide is replaced by a more anodic peptide. (A trace of β^A globin was present in this preparation.) Electrophoresis at pH 6.4 in pyridine:acetic acid:water (10:0.1:90). Chromatography in iso-amyl alcohol:

Table 1 Amino-acid Analysis of Hb-Camden β T13

Amino-acid	Molar ratios of amino-acids	
	Hb-Camden β T13	Expected values from Hb-A β T13
Thr	0.9	1
Glu	3.2	3
Pro	1.9	2
Ala	1.9	2
Val	1.2	1
Tyr	0.8	1
Phe	1.0	1
Lys	1.0	1

Hb-Camden β T13 was hydrolysed with 6.7 N HCl at 110° C for 16 h. The hydrolysate was applied to an amino-acid analyser.

molar ratios of amino-acids as would be expected from Hb-A β T13 (Table 1), indicating the replacement of one glutamine residue by glutamic acid. Two glutamine residues are, however, present in this peptide from Hb-A:

127 131

Hb-A β T13 Glu.Phe.Thr.Pro.Val.Gln.Alu.Alu.Tyr.Gln.Lys

To determine which glutamine residue was substituted, Hb-Camden β T13 was partially digested with carboxypeptidases A and B⁷. Subsequent reaction of the amino-acids released with dansyl chloride, followed by high voltage electrophoresis⁸, showed the presence of Lys,Glu,Ala and traces of Gln and Asp. This result suggested that the more C-terminal, β 131 Gln, was replaced by Glu. Further confirmation was obtained by digesting Hb-Camden β T13 with chymotrypsin, followed by peptide mapping which showed one very strongly staining neutral peptide as well as a number of more weakly staining spots. The strong neutral peptide was hydrolysed with HCl, after which reaction with dansyl chloride followed by high voltage electrophoresis showed the presence of only Glu and Lys and because it was neutral it must have contained equal amounts of these. The neutral character of the peptide indicated that glutamic acid, not glutamine, was present. The peptide Glu-Lys can only have arisen from the C-terminus of β T13 thus confirming that the substitution in Hb-Camden is β 131 (H9) Gln→Glu.

In normal human haemoglobin-A Gln (H9) β 131 forms part of the $\alpha^1\beta^1$ intersubunit contact by coordination with His (G10) α 103 (ref. 9). In a private communication, Dr M. F. Perutz indicated that substitution of the glutamine residue by glutamic acid would be expected to result in formation of a salt bridge between the carboxyl group of the glutamic acid and the imidazole of the histidine. In Hb-A His (G10) α is in a rather secluded position facing the internal cavity on one side and cut off from water on the other sides. Moreover it is probably in contact with the amino rather than the carboxyl side chain of Gln (H9) β . Thus it would be expected to have a low pK and be uncharged at pH 8.6 and pH 6.9. In Hb-Camden, the formation of a salt bridge with the new glutamic acid would raise the pK of the imidazole so that it would now be charged at these pHs and this extra positive charge would compensate the negative charge of the glutamic acid. Such interactions would explain that Hb-Camden has nearly the same electrophoretic mobility as Hb-A at pH 8.6 and pH 6.9.

The functional properties of Hb-Camden in the heterozygote appear to be normal, as no abnormalities in oxygen affinity, haem-haem interactions or Bohr shift could be detected and no clinical symptoms were observed. The effect of this substitution in the $\alpha^1\beta^1$ contact could not be studied further as the patient was no longer available.

Eleven human haemoglobin variants have been reported with substitutions in the $\alpha^1\beta^1$ intersubunit contact¹⁰. Of these six show no abnormal properties, three show only mild abnormalities and only two give rise to haematological symptoms

as a result of molecular instability. These observations are in accordance with data on the normal haemoglobins of other animals, among which more than one third of the residues in the $\alpha^1\beta^1$ contact vary (in contrast to the residues in the $\alpha^1\beta^2$ contact which are virtually invariant)⁹. As yet, however, no amino-acid other than glutamine has been reported at positions β 139 (H9) (ref. 11).

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Paraffin Synthesis in the Oenocytes of the Desert Locust

OENOCYTES are one of the least understood types of insect cells. Several workers have suggested that the oenocytes produce structural lipoproteins for the formation of the cuticle or the paraffins and waxes which are involved in water-proofing the cuticle¹⁻⁴. Electron microscope studies⁵⁻⁹, ligation experiments^{10,11} and biochemical studies¹¹⁻¹³ suggest that the oenocytes rather than the prothoracic glands synthesize the moulting hormone ecdysone. At present, however, there is no direct and conclusive evidence to support either of these hypotheses.

We have investigated the cytology and function of the oenocytes in the desert locust *Schistocerca gregaria*. The animals used were fifth instar female larvae fed on bran and wheat seedlings and exposed to a day of 12 h (35° C) followed by a night of 12 h (25° C). The instar lasts about 9 days. The oenocytes are found only in the peripheral abdominal fat body in association with ordinary fat body cells and with some small "urate cells". The central fat body surrounding the gut contains only ordinary fat body cells. The extensive smooth endoplasmic reticulum which we have observed in the oenocytes suggests that these cells synthesize lipids. We therefore carried out incorporation studies using ¹⁴C-acetate with both types of fat body and with hemodermal cells from the tarsus of the first thoracic

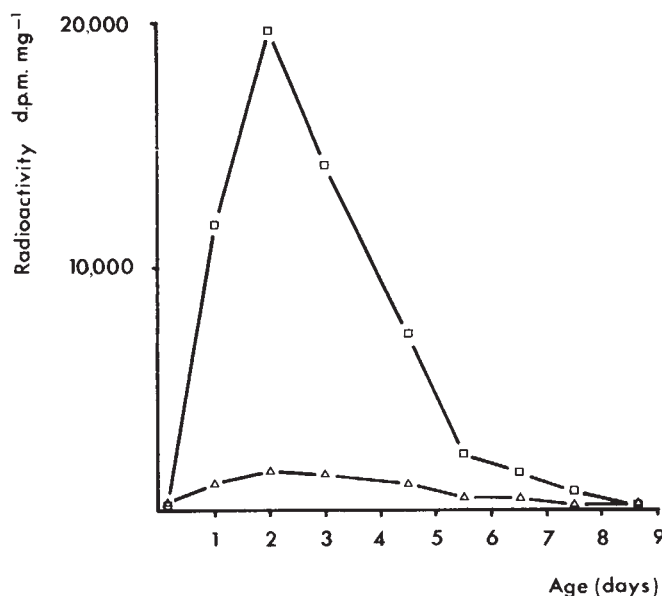


Fig. 1 ^{14}C -labelled lipids from the central yellow fat body during the development of the fifth instar in *Schistocerca gregaria*. Radioactivity (in discharges per minute, d.p.m.) in phospholipids (Δ) and triglycerides ($\square$) per mg tissue (wet weight). The instar lasts about 9 days.

segment. Dissections and incubations were carried out in a buffered Ringer solution (20 mM PIPES-buffer at pH 6.8 containing 125 mM Na^+ , 10 mM K^+ , 2 mM Ca^{2+} , 2 mM Mg^{2+} , 143 mM Cl^- , and 2.5 mM glucose). In each experiment 100 mg of fat body or 4 terga, freed from other tissues, were incubated for 1 h at 35°C in 2.5 ml. of the Ringer containing 1.6 μCi of uniformly labelled ^{14}C -Na-acetate (0.03 μM). After homogenization the lipids were extracted with chloroform-methanol (2:1 v/v)¹⁴, separated by thin-layer chromatography on silica gel G using several solvent systems of differing polarity and identified by comparison with lipid standards. Radioactive spots were located with a plate scanner, removed from the plate and counted by liquid scintillation counting.

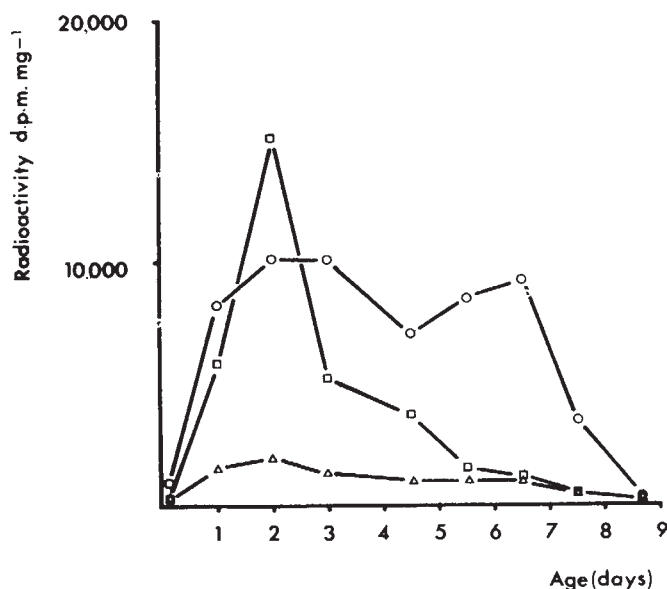


Fig. 2 ^{14}C -labelled lipids from the oenocyte-rich peripheral fat body. Radioactivity (d.p.m.) in phospholipids (Δ), triglycerides ($\square$) and paraffins ($\circ$) per mg tissue (wet weight) determined at various stages of the fifth instar.

Incorporation of the radioactive precursor was followed daily throughout the instar. Figs. 1 to 3 show that both phospholipids and triglycerides were labelled extensively in all three tissues. In both types of fat body the high rates of incorporation into triglycerides confirm previous observations¹⁵ that triglycerides are the principal lipids synthesized by and stored in the fat body of *Schistocerca*. In the oenocyte-rich peripheral fat body, however, there is a third extensively labelled lipid fraction which is absent from the central fat body (Fig. 2). Thin-layer chromatography suggests strongly that these lipids are paraffins because they co-chromatograph in hexane with paraffin standards. Several lipids of unknown nature were also labelled to a small extent. (The data for these small fractions are omitted from Figs 1 to 3.) The hypodermis also synthesizes paraffins (Fig. 3), but the amount is so small that the incorporation of ^{14}C -acetate into paraffins by the entire hypodermis is less than 1% of that simultaneously incorporated by the oenocyte-rich fat body. Thus the synthesis of paraffins must take place primarily in the oenocyte-containing peripheral fat body.

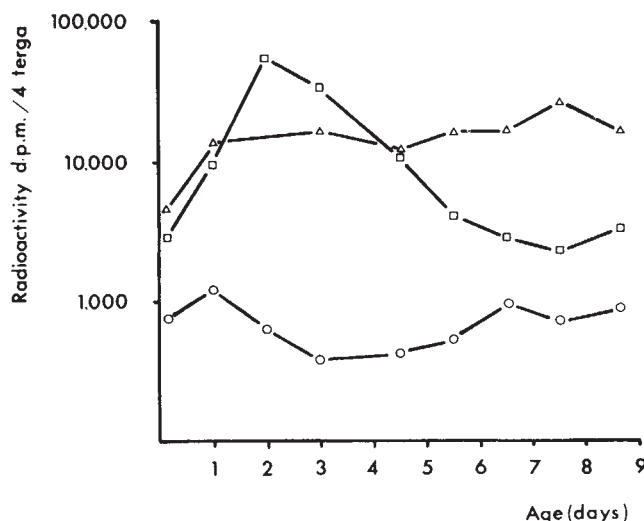


Fig. 3 ^{14}C -labelled lipids from the hypodermis of the tergum of the first thoracic segment. Radioactivity (d.p.m.) in phospholipids (Δ), triglycerides ($\square$) and paraffins ($\circ$) determined at various stages of the fifth instar. Four terga were used in each experiment. Note that the vertical scale is logarithmic by contrast with those of Figs. 1 and 2.

The "paraffin" fraction isolated by thin-layer chromatography was analysed by gas-liquid chromatography on a SE-30 column. Most of the components showed retention times similar to those of the n-alkanes C_{21} - C_{33} with the odd-numbered C_{25} -, C_{27} -, C_{29} - and C_{31} -n-alkanes dominating. Infrared spectroscopy also suggests that these lipids are saturated paraffins.

Our cytological and histochemical studies suggest that the oenocytes are responsible for the synthesis of paraffins. This is supported by a cell separation experiment. Oenocyte-rich fat body from 5-day-old larvae was incubated as above and then placed for 10 min in Ringer solution at 35°C containing 0.05% 'Pronase'. Cell separation was facilitated by repeatedly drawing the tissue sample through a pipette. Centrifugation at low speed (3-5g) for 1 min left most of the fat body cells in a froth at the surface. Most of the "urate cells" remained in the supernatant. The pellet contained an abundance of oenocytes with a few fat body cells and "urate cells". In the pellet the ratio between the radioactivity in the paraffin fraction and in the triglyceride fraction was 13:1 whereas in the intact control tissue the ratio was 2.3:1. This enhancement of the paraffin fraction following partial separation of the oenocytes strongly supports the view that these cells are respon-

sible for paraffin synthesis and that triglyceride synthesis is mainly associated with the ordinary fat body cells.

The paraffins synthesized in the oenocytes must be transported to the cuticle where they make up 40–60% (w/w) of the chloroform-soluble cuticle lipids. Indeed, after *in vivo* incorporation of ^{14}C -acetate, paraffins are the most extensively labelled of the cuticular lipids. From the above results, we conclude that in *Schistocerca* larvae a large proportion of the cuticular lipids is synthesized by the oenocytes and that this must be one of the main functions of these cells. The role of oenocytes in ecdysone synthesis, however, remains uncertain. ^3H -cholesterol injected into locusts without prothoracic glands is converted into α and β -ecdysone (identified by thin-layer chromatography of their trimethylsilyl derivatives¹⁶). At present one cannot exclude the possibility that oenocytes may be responsible for this conversion.

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Suppression of the Cyclic Surge of Luteinizing Hormone Secretion and of Ovulation in the Rat by Δ^1 -Tetrahydrocannabinol

In the rat, a surge of gonadotrophin secretion occurs during the afternoon of the day of pro-oestrus^{1,2}. This induces the resumption of the first maturation division of the oocyte and changes in the follicular wall that culminate in ovulation—the release of the mature secondary oocyte. The cyclic discharge of ovulating hormone, principally pituitary luteinizing hormone (LH), depends on the secretion of LH-releasing hormone (LRH) by the hypothalamus³. LRH secretion, and consequently LH surge and ovulation, can be blocked by several neurodepressive drugs⁴.

Δ^1 -Tetrahydrocannabinol (also known as Δ^9 -tetrahydrocannabinol—the designation Δ^1 corresponds to the nomenclature proposed by Mechoulam⁵) (THC) is the chief psychoactive ingredient of marihuana⁶. It acts on the central nervous system, causing changes in the EEG⁶ and in the metabolism of 5-hydroxytryptamine and catecholamine^{7–10}. The drug also activates the pituitary-adrenal axis¹¹.

We report here an effect of THC on the serum concentration of LH and on ovulation in the rat. Three-month-old Wistar

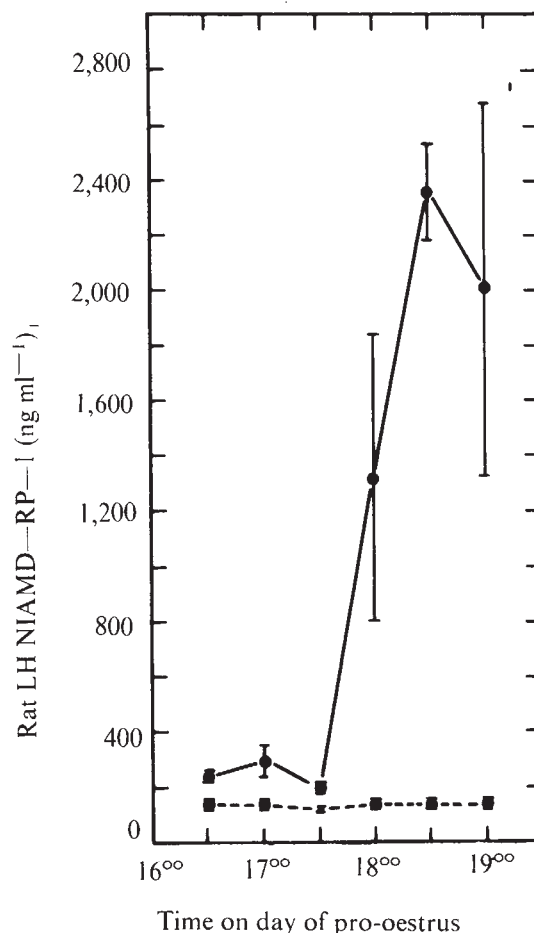


Fig. 1 Effect of Δ^1 -tetrahydrocannabinol (THC) on the concentration of serum LH of pro-oestrous rats. Intraperitoneal injections of THC (1.0 mg per rat each) were given at 1400 and 1600. Control animals received two injections of the vehicle. Vertical brackets indicate $\pm$ s.e.m. —, Control rats; ---, rats treated with THC.

rats weighing approximately 200 g were kept in quarters illuminated between 0500 and 1900. All had shown at least two normal 4-d-cycles, as determined by daily vaginal smears, immediately before the experiment. THC (2 mg per rat, or approximately 10 mg kg⁻¹), divided into two equal doses, was injected intraperitoneally into one group of twenty-four rats at 1400 and 1600 on the day of pro-oestrus. A control group received injections of the vehicle (a Tween-propyleneglycol-water mixture). Between 1630 and 1900, blood samples were drawn by cardiac puncture under light ether anaesthesia from four animals in each group at intervals of 30 min. No rat was bled more than once. The concentration of LH in the serum collected from these samples was determined by radioimmunoassay¹². As shown in Fig. 1, treatment with THC prevented the characteristic afternoon rise in the concentration of serum LH which occurs in the control group.

In another experiment, rats treated with THC on the day of pro-oestrus at the times and dose-levels specified in Table 1, and vehicle-injected controls, were killed the following morning and the oviducts were examined for the presence of shed ova. If ovulation had not occurred, the oocytes retained in enlarged follicles were aspirated and examined microscopically; oocytes devoid of a germinal vesicle and nucleolus were classed as mature.

THC (total dose 2 mg per rat) effectively blocked ovulation (Table 1). In five of the twenty animals in which ovulation was blocked by THC, however, the oocytes entrapped in the follicles were found to have matured. At lower doses (0.4–0.8 mg per rat total dose), the concentration of serum LH was significantly lowered (50% reduction; $P < 0.05$, $n = 41$), but not sufficiently

Table 1 Suppression of Ovulation by Δ^1 -Tetrahydrocannabinol (THC)

THC (mg per rat)*	Treatment		Ovulation	
	Nembutal (mg kg ⁻¹ body weight)†	LH (μ g per rat)‡	No. of rats No. treated	Mean No. ova per ovulating rat
(Vehicle only)	—	—	26/27	10.4
0.4–0.8	—	—	7/8	10
2.0	—	—	1/21	1
—	30	—	0/6	—
—	30	2.5	6/7	12.0
2.0	—	2.5	4/10	8.3

* Divided into two equal doses given intraperitoneally (i.p.) at 1400 and 1600 on the day of pro-oestrus. Oviducts inspected at 0900 the following day.

† Given intraperitoneally at 1400 on day of pro-oestrus.

‡ Given intraperitoneally at 1630 on day of pro-oestrus.

to prevent ovulation (Table 1). All THC-blocked rats ovulated 24 h after the normal time. A similar delay in ovulation occurs in rats after treatment with pentobarbital sodium (Nembutal)⁴.

Treatment with LH, at a dose sufficient to cause ovulation in Nembutal-blocked rats (2.5 μ g per rat), caused only 40% of the animals treated with THC to ovulate (Table 1). So a direct effect of THC on the ovary, in addition to its suppressive effect on secretion of LH, cannot be excluded. Accumulation of THC in corpora lutea after intravenous injection of the drug into mice has been reported¹³. Indomethacin, an inhibitor of prostaglandin synthesis, blocks the ovulatory action of LH at the ovarian level¹⁴. It remains to be seen whether the two drugs act on the ovary in a similar manner¹⁵.

The cyclic surge of LH is particularly sensitive to agents affecting the central nervous system. It cannot be concluded from our experiments that THC has a similar inhibitory action on the steady, "tonic" secretion of LH in either sex. Neither do we know whether the anti-gonadotrophin and anti-ovulatory actions of THC observed are caused by the drug itself or by one of its metabolites¹⁶. Finally, it should be noted that the doses used in these experiments were high (2 mg per rat) compared with the estimated intake by human drug users (probably <1 mg per man), and the route of administration differed.

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Uterine Activity following Intravenous Administration of Oxytocin to the Foetal Sheep

CONCENTRATIONS of oxytocin in umbilical cord blood are high in the newborn human¹ and in several species the foetal pituitary contains large amounts of oxytocin and neural lobe hormone carrier proteins, the neurophysins^{2,3}. If the foetal posterior pituitary is to play a part in labour, however, it must not only secrete oxytocin but this oxytocin must pass from the foetal vasculature to the myometrial cell. No observations have been reported regarding the ability of oxytocin to produce uterine contractions when administered from the foetal side of the placenta.

In five pregnant ewes of gestational age 135–139 days catheters were placed in the foetal dorsal aorta and inferior vena cava and a branch of the maternal uterine vein⁴. In one animal with a twin pregnancy, intravascular catheters were placed in each foetus. A saline-filled balloon was placed in the uterus at the same time to monitor uterine pressure changes. Two days were allowed to elapse before any experiment was performed. Progesterone and oestrogen were measured using antisera donated by Dr G. Abraham. Plasma was extracted with ether and measured by a modification of the method of Abraham *et al.*⁵, but without chromatographic separation. By this method the recovery for progesterone was 90% and 93% for total oestrogens. Plasma cortisol was measured by the competitive protein binding method of Murphy⁶, with a recovery of 87%. No correction has been made for recovery in the values reported.

All intravascular injections were given in 1.0 ml. of physiological saline (0.9% NaCl w/v) infused over 10 s. At the beginning of each experiment a control injection of saline was given into the foetal venous catheter and the uterine pressure observed for a period of 30 min. This was followed by a control injection into the maternal uterine vein catheter. After a further 30 min 100 mU 'Syntocinon' (Sandoz) was administered through the foetal catheter. An hour later the same

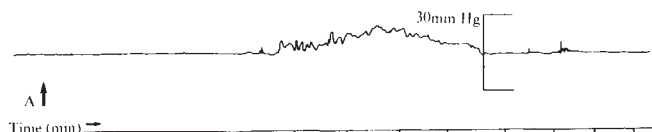


Fig. 1 Uterine pressure recording in response to 100 mU of 'Syntocinon' administered through the foetal inferior vena cava in the pregnant sheep. A marks injection of 'Syntocinon'. Sheep No. 67 on day 141. Foetal cortisol was 8.1 μ g per 100 ml., rising to 12.5 μ g per 100 ml. on the following day. Maternal oestrogens were 0 pg ml.⁻¹ rising to 450 pg ml.⁻¹ on the following day.

Table 1 Effect of Maternal and Foetal Administration of Syntocinon on Uterine Activity in Sheep of Known Gestational Age

Animal No.	Route of administration and dose; single injection of 100 mU syntocinon	Increase in uterine pressure	Latency	Foetal corticosteroids $\mu\text{g } 100 \text{ ml.}^{-1}$	Maternal progesterone ng ml.^{-1}	Maternal total oestrogens pg ml.^{-1}
161 (139)*	Foetal	—		3.6	6.1	0
(139)	Maternal	++	30 s			
(140)	Foetal	—		5.3	7.5	0
(140)	Maternal	++	60 s			
(141)	Foetal	++	2.5 min	7.2	4.7	0
(141)	Maternal	++	30 s			
159 (139)	Foetal	—		3.7	19.8	264
(139)	Maternal	+	2 min			
(140)	Foetal	+	4 min	5.7	17.7	238
(140)	Maternal	+	2 min			
(141)					10.8	370
Birth (142)					1.7	1,132

Data are given for plasma concentrations of maternal oestrogen and progesterone and foetal corticosteroids.

* Gestational age.

dose of 'Syntocinon' was given to the mother. The four animals with single foetuses were tested again the following day and one of these (No. 161, see Table 1) on the third day. Two animals delivered live foetuses within 24 h of the last experiment to test the response to oxytocin. In the other three, live foetuses were delivered by caesarean section after the last experiment. The ewe with the twin pregnancy was only investigated on one occasion and immediately afterwards the lambs were delivered by caesarean section.

Infusion of saline into the foetal or maternal catheter did not produce contractions on any occasion. In two animals with single foetuses, foetal injection of oxytocin produced an increase in uterine pressure at each administration. In the other two animals with single foetuses shown in Table 1, the first injection of oxytocin on the foetal side was without effect. Foetal administration of oxytocin did produce contractions on day 140 in sheep 159 and day 141 in sheep 161. No effect was obtained on day 140 in sheep 161. In each case the appearance of a uterine response to foetally injected oxytocin was associated with a lower maternal jugular plasma progesterone concentration and a higher foetal plasma cortisol than on the occasion when no response was obtained. In the twin pregnancy injection of 'Syntocinon' into either foetal catheter produced contractions. During the 3 h of the experiment, plasma cortisol in one foetus rose from 6.3 to 10.5 μg per 100 ml. and in the other from 3.6 to 5.0 μg per 100 ml. These values suggest that parturition was imminent⁷.

The type of contraction pattern elicited by 100 mU oxytocin injected into the foetus is shown in Fig. 1. There was always a general rise in uterine tone on which were superimposed several small contractions. The latency of the response was 2.5–4 min. Large rhythmic contractions were never seen after the foetal administration of oxytocin but the contraction pattern may be different closer to the time of normal delivery.

The administration of oxytocin to the mother invariably resulted in increased uterine activity (Table 1). Although the contraction pattern was largely similar to that observed after a foetal injection, the following differences were found. After the maternal injection there was a shorter latency to the onset of the contractions; the duration of increased uterine pressure was longer; the increase in pressure was greater and there was more tendency to establish rhythmic contractions. In two animals an increased response to maternal administration of oxytocin was noted on the second occasion the animal was tested.

These results show that 100 mU oxytocin (200 ng) injected into the foetal circulation can stimulate the myometrium near term in the sheep and that the latency of this response is considerably longer than when the same amount of hormone is given directly into the maternal circulation, despite the very

much greater maternal plasma volume. The longer latency of the response to the hormone when given intravenously to the foetus suggests that placental transfer must be slow. Further work is necessary to elucidate the pathway by which oxytocin passes from the foetal blood to cause contraction of the myometrium.

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Chimaeras Obtained by Aggregation of Mouse Eggs with Rat Eggs

TECHNIQUES have been developed which enable mouse eggs differing in age and stage of development to aggregate and form large but apparently morphologically normal blastocysts capable of egg cylinder formation at least^{1,2}. Such chronological regulation has also been demonstrated for the mouse blastocyst by transfer of an isolated 4.5-day inner cell mass to a 3.5-day blastocyst with the subsequent formation of a normal chimaera³. These systems show that chimaeras derived from two components with the same pattern of differentiation, but with a temporal discrepancy experimentally imposed, can develop normally. This suggests that rat and mouse eggs, in which the pattern of differentiation is similar but on a different time scale, might develop successfully when aggregated together. This hypothesis was supported by a pilot study⁴ which has been extended and is reported here.

Eggs were collected from Q strain mice and Wistar rats

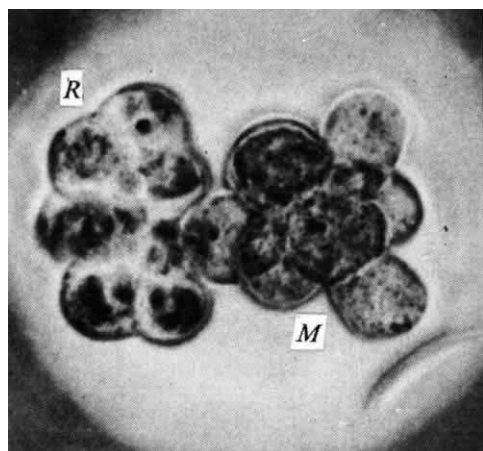


Fig. 1 An eight-cell mouse egg (M) conjoined with an eight-cell rat egg (R).

and cultured in Whittingham's medium⁵. They were prepared for aggregation using routine techniques¹.

Of fifty-nine eight-cell rat eggs paired with mouse eggs at four-cell, eight-cell or early morula stages, thirty-six (61%) aggregated together (Table 1). Of these, sixteen out of fifty-nine (27%) formed blastocysts which appeared morphologically normal. Although total incorporation of both components occurred in ten cases a few cells were excluded from the other six chimaeric blastocysts. The exclusion of some cells is not, however, uncommon in such experimental studies². The remaining twenty, (34%) chimaeric aggregates failed to develop a blastocyst cavity. Whether this was the result of manipulative procedures, culture conditions or a factor inherent in the eggs themselves is not known.

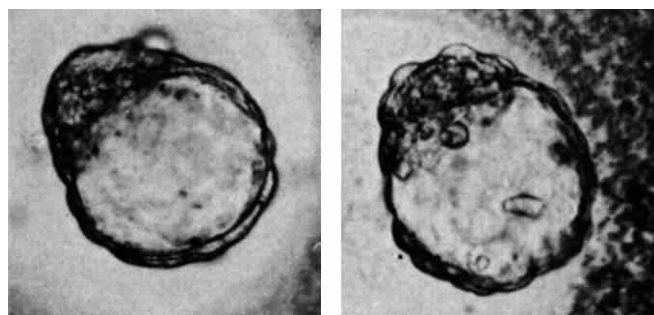


Fig. 2 The blastocysts developed from pairs of mouse and rat eggs similar to those shown in Fig. 1.

Table 1 Development of Conjoined Pairs of Rat and Mouse Eggs

Stages * conjoined	No aggregation	Degree of development Aggregation but no blastocyst cavity formed	Blastocyst formation
8 × 4	1	4	1
8 × 8	20	16	13
8 × EM	2	—	2
Total	23 (39%)	20 (34%)	16 (27%) †

* The first named egg of each pair is the rat egg. † This compares with 42% successful blastocyst formation from asynchronous mouse/mouse aggregates¹. EM, Early morula.

This study shows that the difference in intrinsic rate of development between rat and mouse does not form an obstacle to their aggregation and cooperation in subsequent blastocyst formation. Furthermore, apparently normal blastocysts can be produced when the pairs of eggs conjoined are of differing cleavage stages (Table 1). The remarkably flexible developmental potential of the rodent egg, thus demonstrated, offers an opportunity of following the interaction of genotypically different cell populations during differentiation and, further, the possibility of elucidating aspects of foetal/maternal interactions such as implantation.

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Impairment of Latent Learning in the Rat by a Marihuana Component

NUMEROUS studies in both humans¹⁻³ and animals⁴ indicate that marihuana and its chief psychoactive constituent, Δ^9 -tetrahydrocannabinol (Δ^9 -THC), may have disruptive effects on short term memory. It has been suggested, however, that the memory-disrupting qualities of marihuana in humans may actually be incidental to its effect on normal attentional processes⁵. Furthermore, interpretation of animal investigations in terms of drug-induced memory disruption may be difficult as Δ^9 -THC may alter appetitive motivation⁶. So it is unclear whether performance deficits are a consequence of impaired memory, attention, or motivation.

One way to circumvent possible motivational problems and at the same time study the effects of Δ^9 -THC on attention is to use an experimental paradigm which takes into account both of these factors. In a latent learning paradigm, rats can be pre-exposed to a distinctive environment such as a maze for some time with no food and can be allowed to explore freely. Subjects can then be deprived during an acquisition phase with food introduced to determine whether they benefited from their exploratory experience in terms of some measure such as reduced error scores in comparison to non-exposed controls. If Δ^9 -THC interferes with the ability to attend to significant environmental cues, animals treated with the drug during free exploration may not show any benefit from their familiarization period when tested during an acquisition phase in a drug-free state.

The subjects were thirty-two naive male hooded rats 100–110 d old at the start of the experiment. The apparatus consisted of a seven-unit multiple Y maze with interchangeable steel units. A grey wooden box with hinged top and guillotine door served as a goal box. Individual Y units were arranged so that the sequence of correct choices was LLRRLRL.

The latent learning procedure which was used resembles the type II design identified by MacCorquodale and Meehl⁷ which consists of free exploration followed by reward. After a brief period of handling, all rats were habituated for 2 d to a single Y unit located on the floor for 15 min d⁻¹. After habituation, subjects were randomly assigned

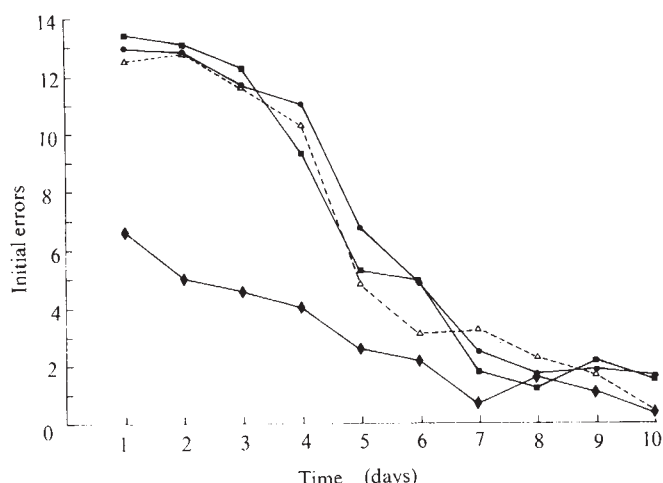


Fig. 1 Effects of Δ^9 -THC on the mean number of initial errors over 10 acquisition days. ●, CON-D; ■, LAT-D; △, CON-ND; ◆, LAT-ND.

to one of four groups, latent learning-drug (LAT-D); latent learning-no drug (LAT-ND); control drug (CON-D); control-no drug (CON-ND). Subjects in the two latent learning groups were exposed to the multiple Y maze for 15 min d^{-1} for 3 consecutive days. Although the goal box was not baited on exposure days, the small glass tray which was to be used as a food receptacle was present. The two control groups received continued exposure to the single Y unit for the 3-d period with goal box conditions identical to those experienced in the multiple Y maze. Thirty minutes before exposure on each of the 3 d the subjects were dosed intraperitoneally with either vehicle or 3 mg kg^{-1} Δ^9 -THC in 1% Tween-80-water.

The choice of a dose of 3 mg kg^{-1} was based on pilot observations which indicated that although this dose was maximally effective in blocking latent learning, no reduction in cul entries occurred. The number of culs entered during exploration is inversely related to the number of errors made during testing⁸. Doses higher than 3 mg kg^{-1} reduced the number of culs entered during exploration. After each exposure subjects were returned to their home cage and allowed access to the food pellets which were to be used in the acquisition phase. During the exploration phase subjects were reduced to 85% body weight. Although the procedure of depriving the animals during exploration may not be desirable because of a possible interaction between drug treatment and deprivation condition, it was used because a pilot study indicated that two 24 h deprivation periods immediately after the last day of exposure were not sufficient to motivate the rats to eat in the goal box.

On the day after completion of exploration, each subject was given four rewarded placements in the goal box, receiving two 250 mg Noyes pellets on each trial. Acquisition was begun on the next day. All subjects received four trials a day for 10 d in the multiple Y maze with intervals between trials of 1 min. Two types of errors were recorded: initial errors, which consisted of a subject making an incorrect turn at a choice point; and retracing errors, which consisted of a subject making the correct turn at the choice point and then retracing and committing an error.

A 2 (latent against control) by 2 (drug against no drug) by 10 (acquisition d) analysis of variance was performed on the mean number of initial errors made during acquisition. These means are plotted across acquisition days in Fig. 1. The most relevant features of this analysis were the highly significant interaction between the latent-control effect with days ($F=2.90$, $d.f.=9,252$, $P<0.005$) and drug-no drug effect with days ($F=2.67$, $d.f.=9,252$, $P<0.01$). The triple inter-

action also reached significance ($F=2.85$, $d.f.=9,252$, $P<0.005$). Comparisons of individual cell means revealed that the group receiving vehicle during maze exposure made significantly fewer errors over the first 5 days when compared with group CON-ND ($P<0.01$). The LAT-D group did not differ from its respective control group, indicating that THC nullified the latent learning effect. The LAT-D, CON-D, and CON-ND groups also did not differ from one another. The incidence of retracing errors was quite low and did not differ among the various groups. A t test indicated that the mean numbers of cul entries made by subjects in the LAT-D and LAT-ND groups were not different, although the number of culs entered each day decreased over the 3 d exposure period.

Although no final conclusions can be drawn on the basis of a single study, our results suggest that rats exposed to the maze under the influence of THC may have attended to fewer of its salient features and therefore learned less about the correct path to the goal. It is also possible that, under THC, erroneous response patterns were established during exploration and these carried over to acquisition. If THC blocks habituation as Brown⁹ has contended, the probability of entering previously explored but incorrect blinds would remain high. In a design of this type one cannot, however, rule out with certainty that the lack of a latent learning effect in group LAT-D could be a result of dissociation brought about by the change in drug state¹⁰ from exploration to acquisition.

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Electrical Method for Controlling Pain

AN instrument capable of producing effective dental anaesthesia has recently attracted some publicity in Britain, although no reports have yet been published. Briefly, a controlled constant current is provided by a 22 V battery, the positive lead being connected to a dental drill, insulated at the handle, and the negative lead attached by a metal clip to the ear of the patient. Current flow is controlled to give an output ranging from 0 to 60 μA as registered on a meter. The instrument, which is of Russian design and marketed in West Germany, is both compact and portable. Preliminary studies on a number of patients suggest that anaesthesia is effective with constant

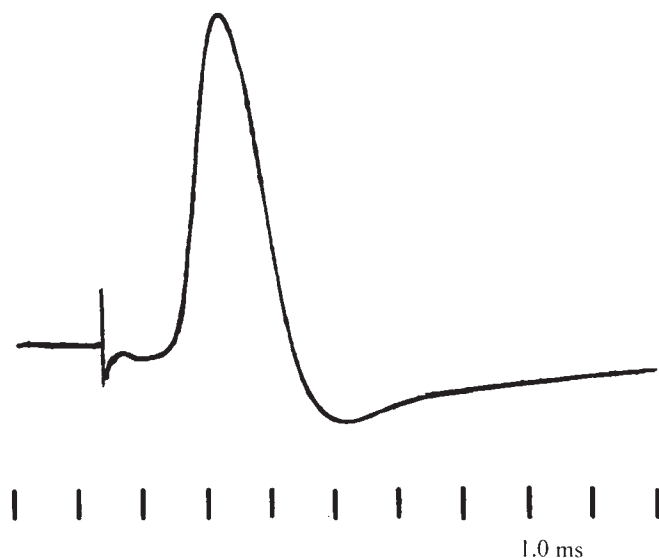


Fig. 1 Compound action potential of frog sciatic nerve. Maximal response with single stimulus 2.5 V, 0.01 ms. Impulses are conducted in all the fibres of the nerve trunk.

currents ranging between 45 and 60 μ A. In several drilling procedures that would have required injections of local anaesthetic in normal practice, the patients did not feel any pain.

There are several ways of inducing blocking of nerve impulses, local anaesthetics, cold, heat and pressure being the more usual agents. For example, below a certain temperature the propagation of the excitatory process is abolished, and various narcotics such as procaine diminish conductivity of the nerve fibres.

To demonstrate the method of anodal blocking, the frog sciatic nerve was stimulated at its distal end by means of a square wave pulse generator and the nerve action potentials, recorded from a proximal pair of electrodes, were displayed on an oscilloscope. The strength of the stimulus was arranged to produce a maximal response in order to excite all the individual fibres (Fig. 1). When the output leads of the instrument were

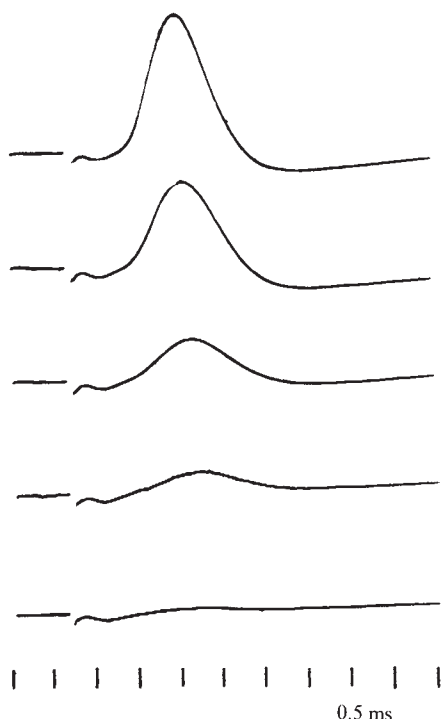


Fig. 2 Changes induced by flow of anodal current. Note progressive reduction in amplitude until action potential is abolished. Conduction of all nerve impulses is blocked.

connected to a portion of the nerve between stimulating and recording electrodes, changes were induced by the flow of anodal current, as shown in Fig. 2. As the strength of the current was increased, there was a progressive reduction in the amplitude of the action potentials with prolongation of conduction times until complete blockade was achieved. The depression was maintained during current flow and persisted for a few seconds after the current was switched off. Full recovery of the action potential was observed after about 10–15 s. This procedure could be repeated many times without any obvious signs of fatigue in the nerve or changes in the form of the records.

The potentials induced in a nerve membrane by an electrical stimulus are well known: the local state of polarization is disturbed and the membrane becomes freely permeable to ions. The depolarized region of the nerve fibre initiates a local flow of current which extends into the adjacent resting membrane. This in turn becomes depolarized and a nerve impulse is propagated along the fibre. The effect of an applied anodal current is to oppose these changes either by restoring the polarized state or by producing hyperpolarization of the nerve membrane. The latter would account for the observed period of after-depression.

Although the concept of anodal blocking of nerve impulses has been evident for many years, its application to the clinical relief of pain is quite new. The apparent success of the method in dental anaesthesia is at least based on sound physiological principles and it may become the method of choice for treatment of children and for very nervous patients or for those with a high degree of sensitivity for pain. It is also possible, with suitable modifications, that the same principle could be applied to other regions of the body, for example, in the treatment of fractures and emergency conditions that usually require the administration of a general anaesthetic.

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Potato Blight Teratogen

LAURENCE¹, in his criticism of Renwick's inference that potato blight is the principal cause of spina bifida and anencephaly, states: "In any case it is likely that one is dealing not with a single teratogen malformation but with one which is multifactorial". This is difficult to parse, but, as multifactorial is now a fashionable word, and as it is important to try and reduce the emotion, to which Laurence objects so emotionally, this word, at least, might be eliminated from meaningful discourse.

All conditions are, to some extent, variable and, to some extent, this variability is reduced in subgroups defined in either genetical or environmental terms. To say "X disease is multifactorial, or polygenic, or complex, or part-genetic" (to use some peculiarly confusing neologisms) is a statement of the same order of informativeness as "Moon dust is made of chemicals" or "mice are made of atoms". It could not be otherwise.

The relationship between multifactorial inheritance and environmental influence is such that multifactorially determined predispositions, of any strength, can be formally irrelevant to the opportunities for environmental modification. In principle there seems no difficulty in eventually breeding a race of men who could smoke with impunity; meanwhile a 95% reduction in the incidence of lung cancer could be achieved in a generation if smokers gave up the habit. There is no incongruity between Renwick's claim of 95% reduction and Laurence's claim of genetic or other factors. Even if some genetic counsellors have presumed to add spina bifida to those disorders which

carry a genetic risk, although—so far as I know—none have given genetic advice to relatives of victims of smoking, this is no reason to suppose spina bifida to be less optional than lung cancer. Nor is there any problem of only one of a pair of twins being affected. Only a minority of smokers develop lung cancer. Nor is there any incongruity in such reckless hormonal gauntlets as *in vivo* pregnancy diagnosis being a substantial contributor to spina bifida.

In the data of Laurence *et al.*², cited by Laurence³ (also personal communication to Laurence, from Laurence, quoted by Laurence), a most detailed and bias-resistant study gave a maximum likelihood estimate of the proportion of spina bifida caused by hormonal trauma, in those exposed to this trauma, of (323-271)/323 or about 15%.

If it is true that about 7% of women, with their general practitioners, have a sufficient joint irresponsibility to the unborn to diagnose pregnancy in this way, then the best estimate of this "factor" is that it is related to almost 1% of cases, or more than one a month. This is, of course, a very vague estimate—the true figure may be anything from none a year, as Laurence asserts, to one or more a week. This is significant in one sense and not in another. As many authors follow Laurence in confusing statistical significance with biological significance, this is another word which might usefully be avoided unless defined when used.

Finally, if I follow the anti-potato letter, Laurence regrets that the statistics are being disturbed by women who get pregnant and fail to eat potatoes. In view of the extensive, considerable, and consistent data on the recurrence risks of these disorders it is difficult to see why a trial should be controlled. It is only necessary for a few hundred women to fail to produce a second affected child on a potato-free diet, or for a few women to have this misfortune while dieting, to clarify this issue, if, indeed, the chemists and experimental teratologists do not get there first.

Fortunately the consequences of less than half the population not eating potatoes for less than a fiftieth of their lives will hardly have any very serious nutritional or economic consequences and it is difficult to see anything but good coming from the uncontrolled non-eating of potatoes in early pregnancy.

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Associations between the Incidence of Neurological Malformations and Potato Blight Outbreaks over 50 Years in Ireland

RENWICK¹ has suggested that anencephalus and spina bifida in man are possibly caused by a specific but so far unidentified teratogen in potato tubers infected by the blight fungus, *Phytophthora infestans*.

The consumption of potatoes per person in Ireland is high and infestation of the annual crop by the blight fungus is also prevalent². Of all the areas so far studied on a community basis Belfast and Dublin have the highest frequencies of anencephalus³. A similar geographical variation probably exists with regard to the incidence of spina bifida. In 1957, McKay⁴ presented a retrospect of 50 yr (1907–1956 inclusive) of out-

Table 1 Analysis of Variance of the Association Between the Annual Incidence of Anencephalus in Births reported from the Rotunda Hospital, Dublin, 1908–1957, and the Frequency of Blight Outbreaks in the Irish Republic during the Previous Year after allowing for Secular Trend.

Source of variation	Degrees of freedom	Sums of squares	Mean square	<i>f</i> ratio value
Linear trend	2	312.70	156.35	—
Linear trend and blight	3	313.05	104.35	—
Extra attributable to blight	1	0.35	0.35	Not significant
Residual	47	38.55	0.85	—
Total	50	351.60		

$$y = 1.075 (\pm 0.347) + 0.044 (\pm 0.009) \times \text{time} - 0.0025 (\pm 0.004) \times \text{blight frequency}.$$

breaks of potato blight in the Irish Republic. Estimates of the number of outbreaks recorded by the end of June during each year were based on specimens collected by potato inspectors and other local officers, and blight infestation was verified by microscopic examination. We have just completed some studies of epidemics of anencephalus and spina bifida in Ireland^{5,6} based on data reported from the three main Dublin maternity hospitals for 1900 to 1965.

We present here some findings of a multivariate analysis correlating secular trends in the annual incidence rates of neurological malformations observed at the largest Dublin maternity unit, the Rotunda Hospital, and McKay's data on potato blight outbreaks.

We found that the annual malformation data can be adequately represented by a linear regression model; there has been a statistically significant linear increase in the incidence of anencephalus ($f_{2,48} = 192.94$; $P < 0.001$) and of spina bifida ($f_{2,48} = 73.18$; $P < 0.001$) over the 50 yr. We therefore tested the null hypothesis that there was no association between the annual incidence of anencephalus and the frequency of blight outbreaks recorded by the end of June of the previous year after allowing for the secular trend. A similar null hypothesis was examined with respect to spina bifida. Tables 1 and 2 show the fit of the model

$$y = \beta_0 + \beta_1 x_1 + \beta_2 x_2$$

to the anencephalic data and the spina bifida data separately, where y = estimated annual malformation incidence, x_1 = time, x_2 = number of blight outbreaks recorded by the end of June of the previous year, β_0 = constant and β_1 , β_2 = estimated regression coefficients with standard errors in brackets.

Both analyses of variance show that the extra variation accounted for by taking into consideration a knowledge of the frequency of blight outbreaks is very small and does not significantly affect estimation of the incidence of either anencephalus or spina bifida based on information relating to secular trend alone. The regression coefficients (β_{2s}) associated with the blight factor are not significant.

Our findings must be interpreted in the light of certain

Table 2 Analysis of Variance – Spina Bifida

Source of variation	Degrees of freedom	Sums of squares	Mean square	<i>f</i> ratio value
Linear trend	2	241.52	120.76	—
Linear trend and blight	3	242.66	80.89	—
Extra attributable to blight	1	1.14	1.14	Not significant
Residual	47	78.07	1.66	
Total	50	320.73		

$$y = 1.542 (\pm 0.493) + 0.022 (\pm 0.013) \times \text{time} - 0.0045 (\pm 0.005) \times \text{blight frequency}.$$

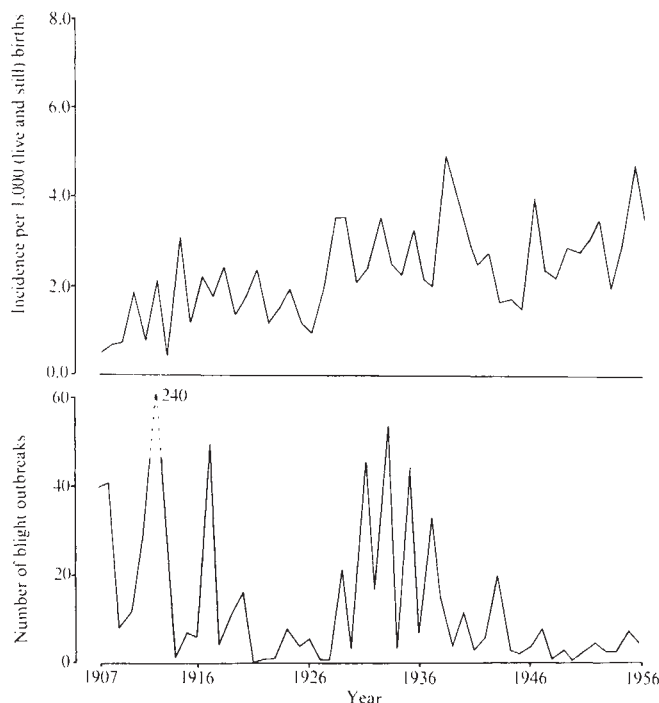


Fig. 1 Annual incidence of anencephalus per 1,000 (live and still) births reported from the Rotunda Hospital, Dublin (upper graph) and the total number of potato blight outbreaks recorded by the end of June of each year in the Irish Republic (lower graph) between 1907 and 1956.

limitations in the two sets of data correlated. Although the malformation data were for hospitals and not the community, as detailed elsewhere⁶ we believe that the secular trends observed are genuine and not an artefact. By using McKay's data to test Renwick's hypothesis we assume that the number of potato blight outbreaks is correlated with the proportion of blighted tubers marketed during the ensuing months, which in turn is associated with the amount of such diseased vegetables consumed by expectant mothers in Dublin. No valid agricultural or other data are available adequately to examine this sequence of events.

Another criticism, namely that McKay's figures relate to the whole of the Irish Republic and not to Dublin only, probably is not too important as a substantial proportion of this country's annual harvest is eaten by the 20% of the total population resident in the capital city. With these provisos, however, our analysis provides no evidence of any linear correlation between the incidence of anencephalus or the incidence of spina bifida and the frequency of blight outbreaks in the previous year. The Irish data thus appear to fit less well with the blight hypothesis than, for example, the further data for Scotland⁷ and England and Wales⁸ which Renwick has published. Whether this is because of the high prevalence of blight almost every year in Ireland—resulting, for example, in an above average number of outbreaks which have little additional effect on a cycle of human development affected by very small amounts of a teratogen—or for other reasons is not known.

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Oxygen as a New Factor controlling Reproductive Growth

It is well recognized from short term experiments that the photosynthetic and vegetative growth rates of plants with high rates of photorespiration, the so-called C_3 or inefficient plants, are elevated 50 to 100% by sub-atmospheric concentrations of O_2 , while those of plants with low rates of photorespiration, the so-called C_4 or efficient plants, is essentially unaffected^{1,2}. The long term effect of O_2 concentrations on vegetative and reproductive growth of either C_3 or C_4 plants has not, however, been reported.

We confirm the observation that vegetative growth in soybean (C_3) and sorghum (C_4) is increased by exposure to sub-atmospheric O_2 concentration throughout most of the growth cycle. A previously unrecognized role of O_2 , however, which is uniquely associated with reproductive growth, occurs in both C_3 and C_4 plants with greater than the 15% of O_2 required for normal seed development. This observation provides a new approach for elucidation of factors controlling reproductive development and translocation. New O_2 reactions may be involved, and the results may be of significance regarding evolution and ecological distribution.

Table 1 Effect of Sub-atmospheric Concentrations of O_2 on Wye Soybean Growth

	5	10	15	21
Dry wt (g)				
Leaves	44.2	20.7	8.2	9.2
Stems	25.5	14.4	3.6	5.0
Pods	7.6	12.9	5.8	8.8
Seeds	0	1.1	12.8	21.5
Roots	18.3	14.8	3.4	2.9
No. of fully developed pods	0	11	64	105
No. of undeveloped pods	804	221	35	6
No. of developed seeds	0	12	125	235

Measured at 83 d of age and exposed to the gas phase indicated for 14–83 d. Each datum is the average of two plants grown in separate chambers. Plants were grown with their aerial portion contained in chambers of 'Lucite' acrylic resin of 145 l capacity with urethane foam gaskets around the stem entrance and purged with air or a synthetic gas mixture at 15 l min⁻¹, whilst the root was exposed to air. CO_2 and O_2 contents of the chambers were adjusted to provide 0.03% CO_2 and the desired O_2 concentration and analysed by gas chromatography. Gas mixtures were prepared by dilution of air with N_2 and addition of CO_2 . The temperature inside the chambers was 30° C by day and 21° C by night; light intensity 1.47 cd cm⁻² (4,300 ft. c); day length 12 h. Plants were grown in 18 cm pots containing a 50/50 mixture of 'Jiffy Mix' and sand and were watered daily with Hoagland's nutrient solution.

Results of an experiment in which the aerial portions of soybeans were exposed to 5, 10, 15, or 21% O_2 from 14–83-d plants are recorded in Table 1. The final dry weight of the aerial portion of the plant was increased 74%, by 5% O_2 as against 21% whilst a greater increase (631%) was found for the root. The most striking effects of O_2 concentration were on the morphology and reproductive character of the plants (Figs 1 and 2). There is an increase in lateral development, diameter of stems, and leaf thickness. Some delay in senescence occurs, while the time of flowering is essentially unchanged. The number of fully developed pods and seeds is decreased by all sub-atmospheric levels of O_2 so that at the time of normal maturation there are neither completely developed pods nor seeds for plants exposed to 5% O_2 , and only 53% as many

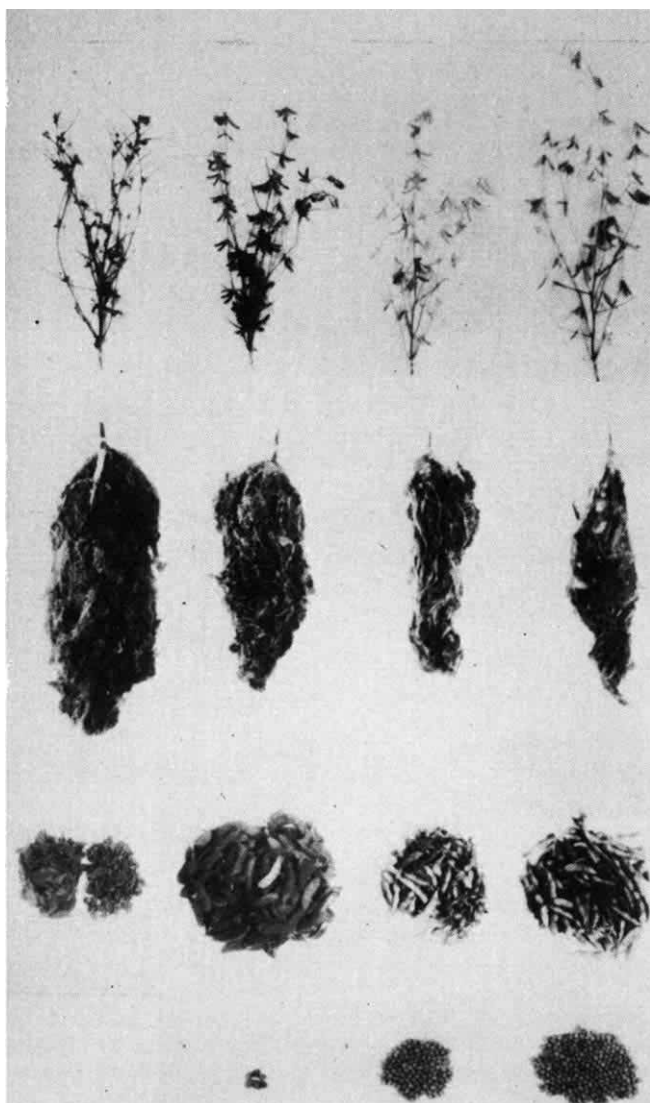


Fig. 1 The effect of sub-atmospheric concentrations of O_2 on growth and development of various plant parts of soybeans; *a*, aerial-portion-defoliated; *b*, roots; *c*, pods; *d*, seeds. (See Table 1 for procedure.)

developed seeds in 15% O_2 . On the other hand, the total number of pods is increased by low levels of O_2 . With 5% O_2 over 800 pods per plant are produced, which is equivalent to 7.24 times the number obtained with plants grown in air (21% O_2). Similar results have been obtained in four separate experiments with levels as low as 1.4% O_2 . Results obtained with synthetic air made by mixing O_2 , N_2 , and CO_2 were identical to atmospheric air. Although 5% O_2 delays senescence by about 30 d, there is no increase in mass of either pods or seeds of plants harvested at 112 as opposed to 83 d. No permanent photosynthetic metabolic changes were found; for example, the CO_2 compensation point of leaves of soybean plants grown under 5% O_2 was essentially normal when measured in air. A preliminary experiment with wheat, another C_3 plant, indicated similar inhibition of seed development by sub-atmospheric concentrations of O_2 .

Exposures of soybeans to 5% O_2 during only certain specific stages of development indicated two critical periods in which O_2 concentration affected reproductive growth. Plants treated from 14–35 d of age, the period of rapid vegetative growth up to mid-flowering, produced large numbers of shortened pods which contained an average of about one seed per pod

which was larger than normal. Plants treated from 35–50 d, the period of early seed development, produced large numbers of pods of normal size, but the pods contained only incompletely developed seeds. Late exposures from 50–83 d, the period of pod filling, did not affect pod development but inhibited seed development. Thus early exposures seem to arrest pod development while mid-term and late exposures arrest only seed development.

In sorghum the number of fully developed seeds is also decreased by all levels of sub-atmospheric concentrations of O_2 whereas the morphology, time of flowering, and dry weights of the aerial portion and roots are not significantly altered (Table 2). Similar results have been obtained in two separate experiments.

Table 2 Effect of Sub-atmospheric Concentrations of O_2 on Martin Milo Sorghum Growth

	% O_2			
	5	10	15	21
Dry wt (g)				
Leaves and stems	57.5	47.6	53.2	66.7
Seed head	5.9	8.5	9.1	16.8
Seeds	0	1.6	1.7	7.7
Roots	38.2	41.1	30.9	43.2

Measured at 168 d of age with aerial portion of the plant exposed to indicated gas phase from 67–168 d. (See Table 1 for experimental procedures.)

By contrast with the beneficial vegetative growth results previously obtained under sub-atmospheric O_2 concentrations in short term studies on C_3 plants¹, our long term studies demonstrate that O_2 concentration is a key factor controlling reproductive growth and the use of O_2 limitation to increase the efficiency of photorespiratory plants is ineffective for seed producing crops. This control role of O_2 requires higher levels of O_2 than mitochondrial respiration. The rate of dark respiration in soybean leaves^{3,4} is the same at 1–3% and 21% O_2 while our results demonstrate that the optimal level of O_2 required for maximal reproductive processes approaches, or even exceeds, atmospheric concentration and reproductive growth may even be favoured by super-atmospheric O_2 concentrations. The nature of the O_2 receptor involved in reproductive growth is not yet identified although systems such as ascorbic acid oxidase and flavoenzyme oxidases are known

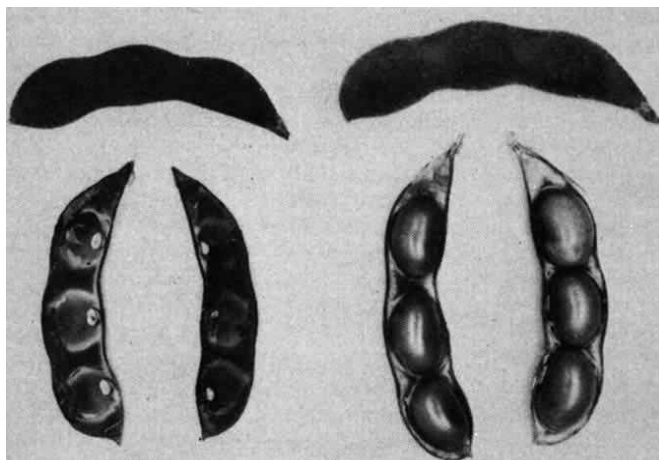


Fig. 2 The effect of sub-atmospheric O_2 on seed and pod development of soybean exposed to 10% O_2 from 14–83 d in comparison to 21% O_2 (air).

to require higher O_2 concentrations than the cytochrome systems of dark respiration⁵. On a physiological basis it seems that an interaction of O_2 with the translocation of photosynthate to the reproductive sinks such as the seed occurs and this may be mediated by an effect of O_2 on the amount or specific activity of endogenous hormones such as auxins, gibberellins, cytokinins, or ethylene. The ability of exogenous hormones to overcome the effect of low O_2 is being tested. It is also noted that the root becomes a more effective sink as the reproductive parts become less effective.

The Earth's atmosphere has altered considerably during its evolution, the O_2 concentration increasing from zero to its present 21%; our data suggest that plants reproduced by seeds may not have evolved until a later stage when the build-up of O_2 in the atmosphere was close to its present proportion.

The requirement for high amounts of O_2 for normal reproductive growth may have ecological significance. At high elevations the amount of O_2 is substantially decreased from that occurring in air at sea level. Seed yields of plants grown on elevated plateaux may be decreased (Allred, R. C., personal communication). On the other hand, vegetative growth and root growth may be improved.

Our observation permits a new experimental approach to elucidate factors controlling plant reproductive development. The occurrence of the O_2 effect in plants with high and low rates of photorespiration suggests that it may be common to a large number of higher plants. Elucidation of its mode of action may provide information useful in increasing seed yield of grain or alternatively vegetative growth of forage or root crops.

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Increased Yield through Correction of Sulphur Deficiency in Ryegrass exposed to Sulphur Dioxide

SULPHUR dioxide (SO_2) in the air at high concentrations may be injurious to higher plants: at low concentrations it is not injurious. On the basis of experimental exposures¹, a concentration of about $429 \mu g SO_2 m^{-3}$ (0.15 p.p.m.) has been widely accepted as a threshold below which a range of higher plants would not be injured even during prolonged exposure. The dividing line between injurious and non-injurious concentrations is not well defined because plants vary in their susceptibility to injury according to the species, stage of growth, environmental factors, such as light intensity, humidity, water supply, and the duration of the exposure at a given concentration². The injurious effects of SO_2 have been investigated extensively but much less attention has been given to its effects at non-injurious concentrations. This aspect deserves

further study because in some circumstances the effects of SO_2 are known to be beneficial. Thus, it has been observed that (i) the rate of photosynthesis in lucerne, as measured by the uptake of carbon dioxide, was increased by exposing the plants to air containing $486 \mu g SO_2 m^{-3}$ for 66 h (ref. 3) and (ii) the yields of dry matter of several species growing in solution culture were enhanced when the shoots were exposed for 9 to 20 days in air containing SO_2 , labelled with ^{35}S , at 200 and $500 \mu g m^{-3}$; ^{35}S appeared in both organic and inorganic fractions of the plant sulphur⁴. The fact that SO_2 can be utilized after absorption through the shoots may be of widespread significance in crop nutrition in industrialized countries. Indeed, we have found recently⁵ that when ryegrass was grown on sixteen soils from England and Wales in a controlled environment cabinet with air filtered to remove SO_2 , the plants developed sulphur deficiency on all soils except one; deficiency has not been encountered in crops growing on these soils in the field. The inputs of sulphur from the air and rainfall to plants and soils under field conditions are not known with certainty, but in rural areas in Britain the air near the ground contains on average $50 \mu g SO_2 m^{-3}$ (ref. 6) and for urban areas the average is slightly above $100 \mu g m^{-3}$ (ref. 7).

We report here the results of an investigation into the effects of prolonged exposure to SO_2 on ryegrass (*Lolium perenne*) variety S23, when grown on a soil known to be unable to meet fully the plant requirement for sulphur⁸.

The experimental soil, which was taken from an area under grass/arable rotation at the Grassland Research Institute, has been described elsewhere⁸. Ryegrass was sown in sixteen polyethylene-lined pots containing 6.26 kg soil (oven dry basis) which received additions of nutrients as follows: 150 p.p.m. nitrogen (as $Ca(NO_3)_2$); 20 p.p.m. phosphorus (as $CaH_4(PO_4)_2$ plus KH_2PO_4); 5 p.p.m. magnesium (as $MgCl_2$). Eight pots of soil received 61 p.p.m. potassium either as KCl, KH_2PO_4 plus K_2SO_4 to give 10 p.p.m. added sulphate-S, while the remaining eight pots (without added sulphate-S) received the same amount of potassium simply as KCl plus KH_2SO_4 . Initially, all pots were housed in a 'Saxcil' controlled environment cabinet with air filtered through activated charcoal to remove SO_2 (cabinet 1). After emergence, the seedlings were thinned to seven per pot, and the surface of the soil was covered with a 1 cm layer of opaque polyethylene granules to minimize evaporation. The pots were weighed at 2-day intervals and distilled water was added to bring the soil to 75 cm water tension (about 1/3 bar). Cumulative water losses were computed to provide an estimate of transpiration. The plants were grown at $23^\circ/18^\circ C$ day/night temperatures with light intensity of $110 W m^{-2}$ and a 16 h photoperiod. Air, with supplementary CO_2 to maintain a level of 300 p.p.m., was pumped into the cabinet after filtering and was continuously circulated; there were six apparent air changes per hour, the flow rate across the plants being about $20 cm s^{-1}$. At 25 days after sowing, one set of pots, comprising four with and four without added sulphate-S, was transferred to another 'Saxcil' cabinet set to give the same environmental conditions as in cabinet 1, except that SO_2 was added to the air at the inlet at a controlled rate (cabinet 2). The concentration in the cabinet was measured each day, usually on two occasions, by a modification⁹ of the West and Gaeke method. The SO_2 flow rate was adjusted so that the mean concentration in the cabinet was $131 \mu g m^{-3}$. The air in cabinet 1 contained no detectable SO_2 .

The ryegrass was harvested at 17 days after the pots were divided between the two cabinets (harvest 1), and again at 38 days (harvest 2) and 59 days (harvest 3). At the first two harvests the shoots were cut at 2.5 cm above the soil, but at harvest 3 they were cut as close to the surface of the soil as possible and the roots were separated from the soil by sieving and washing. After harvests 1 and 2 nutrients were applied again at the original rates.

In cabinet 1 the plants that were grown without added sulphate-S became deficient in S after the first harvest. They were paler green than those grown with added sulphate-S and

Table 1 Yield and Sulphur Content of Perennial Ryegrass Grown in Filtered Air Containing Nil or 131 $\mu\text{g SO}_2 \text{ m}^{-3}$

Cabinet No.	SO ₂ added to air (μg m ⁻³)	Sulphate-S added* (p.p.m.)	Shoot dry weight † and S content ‡						Root dry weight † and S content ‡	
			Harvest 1		Harvest 2		Harvest 3		Harvest 3	
			Dry weight (g)	S content (%)	Dry weight (g)	S content (%)	Dry weight (g)	S content (%)	Dry weight (g)	S content (%)
1	Nil	Nil	11.7	0.26	11.5	0.12	8.7	0.08	8.5	0.11
		10.0	11.2	0.28	16.2	0.31	19.3	0.28	8.7	0.16
2	131	Nil	10.0	0.34	14.7	0.31	14.0	0.17	8.3	0.13
		10.0	10.4	0.37	15.5	0.40	16.2	0.35	7.5	0.17
Standard error (12 df) ±			0.31	0.009	0.67	0.013	1.08	0.015	0.50	0.007

* Addition per pot on each of three occasions, namely before sowing and after harvests 1 and 2. † Dry weight per pot. ‡ Dry matter basis.

were finally distinctly chlorotic. At harvests 2 and 3 the dry weight of the shoots was reduced by 29 and 55% respectively compared with that of plants grown with added sulphate-S; the dry weight of the roots was unaffected (Table 1). In the cabinet with added SO₂ (cabinet 2) there was no evidence of sulphur deficiency, and the dry weights of the shoots and roots of plants grown with and without added sulphate-S were not significantly different.

Results of analysis for total S in the plants using methods described elsewhere⁸ are shown in Table 1. The concentration in the shoots of plants that were grown with added sulphate-S did not differ between harvests in either cabinet. But the concentration in the plants that were grown without added sulphate-S declined between harvests. In cabinet 1 the total decline was about 70%, and the concentration at harvests 2 and 3 was below the range of 0.15–0.20% which has been suggested as "critical", that is, just deficient for maximum growth^{5,10}. It is notable that the input of SO₂ to the plants in cabinet 2 prevented a decline in S concentration in the shoots between harvests 1 and 2, and that between harvests 2 and 3 it maintained the concentration close to the critical value. In fact, at harvest 3 the S content was, in terms of concentration, twice and, in terms of total amount, three times that of the shoots of corresponding plants in cabinet 1.

The beneficial effects of SO₂, as shown by increases in yield and S content of perennial ryegrass when grown with an inadequate supply of S to the roots, are in marked contrast to a recent report¹¹ that exposure to SO₂ depressed yield of the same ryegrass variety grown on a soil, the sulphur status of which was not specified. But the fact that the plants were exposed to higher doses (191 $\mu\text{g m}^{-3}$ for 182 days and 343 $\mu\text{g m}^{-3}$ for 63 days) than in our work may provide some explanation for the different effects. The large amounts of nitrogen added and the cutting treatment that we used simulated more closely the conditions under which high-yielding grass is grown as an agricultural crop in Britain. Although the SO₂ concentration that we used may on occasions be found in agricultural areas, it greatly exceeds the 50 $\mu\text{g m}^{-3}$ measured in areas where most of the grass crop is grown. This, and the fact that SO₂ did not depress yields, even when sulphate-S was added to the soil (as in the field through rainfall), suggests that present concentrations near the ground are not injurious to ryegrass in most areas.

Finally, the effect of SO₂ on transpiration requires comment because it has been suggested that, in Britain, many crops exposed to "ambient SO₂ levels" have an increased rate through decreased stomatal resistance¹². In our investigations, the estimated amounts of water transpired were 10.8 and 11.2 kg per pot in cabinets 1 and 2, respectively. These amounts, which are equivalent to 229 and 234 g water used per g of dry matter produced (see Table 1), do not support the suggestion

that SO₂ has a significant effect on transpiration rate, at least in ryegrass.

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Isomerization of γ -BHC to α -BHC in the Environment

BECAUSE of the wide use of the persistent insecticide benzene hexachloride (BHC), its residues have been found in soils, plants, animals and foods. From the viewpoint of environmental toxicology, the principal residue problem of BHC is that particular isomers, such as α and β , but not the active γ isomer, accumulate in food^{1,2}.

Because the insecticidal γ isomer degrades relatively fast for a chlorinated hydrocarbon insecticide, especially in anaerobic conditions such as an aquatic soil environment^{3–5}, accumulation of α and β isomers has been ascribed to selective metabolism by biological systems, an analogous explanation to that proposed by Ishida and Dahm⁶ for houseflies. MacRae *et al.*⁷, however, showed that in unsterilized soil the persistence of α , β , γ , and δ isomers was essentially the same.

Newland *et al.*⁸ found both α and δ -BHC in aquatic sediments incubated with pure γ -BHC. These workers have attri-

buted the phenomenon to the differential thermodynamic stabilities among the BHC isomers—chemical transformation of γ isomer to more stable α , δ and β isomer.

To study the metabolic fate of γ -BHC in the environment we incubated highly purified γ -BHC with aquatic sediments under simulated natural conditions and with a soil microorganism under laboratory conditions. Non-radioactive γ -BHC used throughout this study contained 0.0017% of impurity as α -BHC. The radioactive ^{14}C - γ -BHC was purchased from Amersham and Searle Corp., and further purified on a thin-layer chromatographic system until no impurities could be detected by an autoradiographic assay.

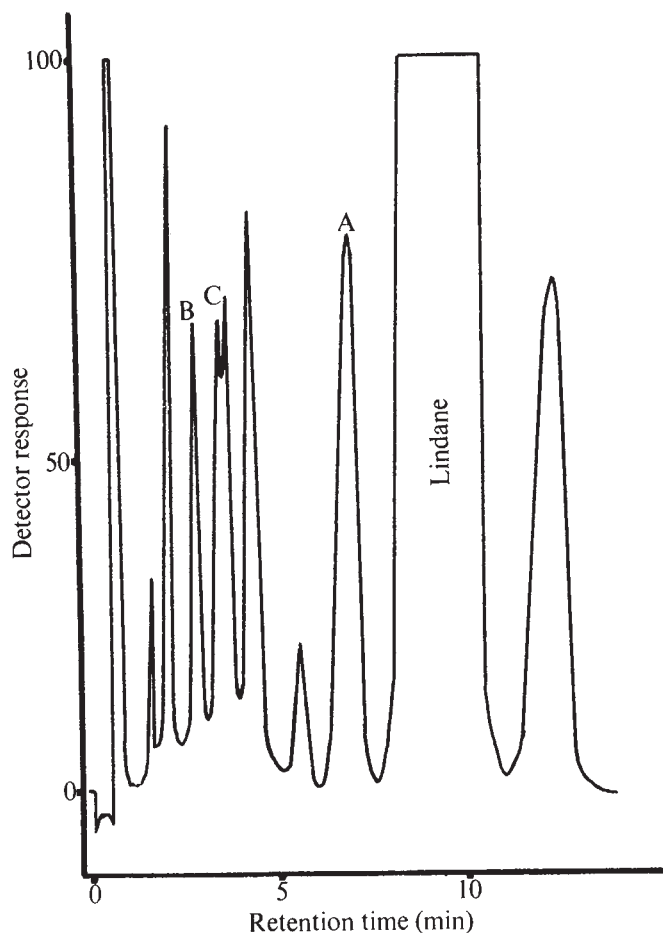


Fig. 1 Sediment from Pearl Harbour, Hawaii, was incubated with γ -BHC for a month. The sediment was extracted, purified, and analysed on a Varian Gas Chromatograph 1800 Series and with an electron capture (^3H) detector: QF1, 3.2 mm $\times$ 1.3 m column at 170°. C, A, α -BHC; B, γ -BTC; C, γ -PCCH (lindane= γ -BHC).

The aquatic sediments (pH, 7.8; total S, 0.091%; SO_4 , 367 p.p.m.; organic matter, 3.4%; sand, 36%; silt, 38%; clay, 26%) were taken from Pearl Harbour, Hawaii, in July 1971, with ambient seawater from a depth of approximately 10 foot. Non-radioactive γ -BHC (100 mg) in 5 ml. of acetone was added to the sediment (approximately 300 g) with 200 ml of seawater. The slurry was transferred into a 600 ml clear, narrow neck medicine bottle which was topped up with seawater. The bottle was sunk to the bottom at the collection site, and was kept there for 30 d.

A portion of the sediment (23.8 g wet weight) was extracted with a 1:1 mixture of chloroform-acetone. A portion of extract was then purified on a 'Florisol' column, 5 $\times$ 65 mm eluting with hexane/ethyl ether (85:15). The results of gas chromatographic analyses (Fig. 1) clearly indicated that the content of α -BHC drastically increased. From the sediment sample (23.8 g)

7.2 μg of α -BHC was isolated (determined by GLC peak areas), a quantity $\times 4$ that found in the original quantity of γ -BHC, even though only a fraction (1/12) of the sediment was extracted. Total recovery of γ -BHC from the sediment was 40%. When untreated Pearl Harbour sediment was extracted and checked on GLC, no peaks were found in the areas with the same retention time as α -BHC (Fig. 1).

For the laboratory study a strain of *Pseudomonas putida* was used. A 48-h-old culture of *P. putida* (2 l) in a mannitol base medium⁹ was incubated with 15 mg of γ -BHC and 100 mg of NAD for 4 weeks. Concurrently small scale experiments were conducted using 10 nm of ^{14}C - γ -BHC and 10 ml of a 24-h-old culture of *P. putida* in base medium with and without the addition of NAD (0.2 mg ml⁻¹). The large scale incubation of γ -BHC with *P. putida* yielded approximately 70 μg of α -BHC in a pure form (90% recovery of γ -BHC). This is more than forty times the amount of α -BHC present as impurity in the culture. Examination of the purification product on mass, GLC and thin-layer chromatographic analyses established it as pure α -BHC.

In the small scale experiment *P. putida* was found to produce normally γ -PCCH (γ -pentachlorocyclohex-1-ene) from γ -BHC, but was found to be capable of producing α -BHC on incubation with NAD. The extent of α -BHC formation was 3% in these experimental conditions. Another principal metabolic product accompanying α -BHC was γ -BTC (γ -tetrachlorocyclohex-1-ene), the same end product as Tsukano and Kobayashi¹⁰ recently found in the paddy soils. The extent of γ -BTC formation under identical conditions was 19%. Recovery in these microbial experiments was greater than 90%.

These results demonstrate that α -BHC can be isolated from the environment in quantities larger than those expected as the basis of selective accumulation of impurities from the starting product alone. The cause of the transformation of γ -BHC to α -BHC in the environment could be microbial, for laboratory studies have shown that a microorganism could indeed convert γ -BHC to α -BHC particularly when the medium is enriched with NAD. More direct evidence is, however, needed to confirm this point.

The significance of this work is that we have demonstrated for the first time that microorganisms in pure culture can isomerize γ -BHC. As there is no document to indicate the presence of this type of isomerization mechanism in the chemical or biochemical fields, further studies on the isomerization process are warranted.

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Earthworms and Seeds

EARTHWORMS move seeds^{1,2} and viable seeds have been reported in worm casts^{3,4}. The causes of loss of seeds from the soil are seldom identified^{5,6}.

In our first experiment fourteen plant species were selected to provide a range of sizes and shapes of seed (Table 1). Petri dishes were prepared with filter paper to which was added 6 ml. of deionized water and the seeds. One specimen of *Lumbricus terrestris* (minimum fresh weight 3 g) was introduced into each dish. The dishes were kept in darkness at laboratory temperatures for 18 h, after which the worms were transferred to fresh dishes, and the seeds remaining in the original dishes counted. After a further 72 h the seeds in casts were recovered and counted.

Table 1 Fate of the Seeds of the Fourteen Species

	Percentage of offered seed ingested by <i>L. terrestris</i>	Percentage of ingested seeds recovered from cast	Percentage of ingested seeds not recovered
<i>Poa annua</i> L.	60	28.3	71.7
<i>Agrostis tenuis</i> Sibth.	50	0.0	100.0
<i>Poa trivialis</i> L.	40	41.0	59.0
<i>Digitalis purpurea</i> L.	32	53.1	46.9
<i>Bellis perennis</i> L.	17	58.8	41.2
<i>Trifolium repens</i> L.	16	62.5	37.5
<i>T. pratense</i> L.	15.5	48.3	51.7
<i>T. incarnatum</i> L.	13	65.0	35.0
<i>Dactylis glomerata</i> L.	10	80.0	20.0
<i>Trifolium striatum</i> L.	7.5	66.7	33.3
<i>Sinapis alba</i> L.	6	66.7	33.3
<i>Festuca pratensis</i> Huds.	4	50.0	50.0
<i>Lolium perenne</i> L.	3	66.7	33.3
Spring barley <i>cv.</i> Deba abed.	0	—	—

Results are for twenty replicates, each of ten seeds, for each species.

L. terrestris ingested seeds, but there was wide variation between species, from 0% for barley to 60% for *Poa annua* (Table 1). None of the ingested grains of *Agrostis tenuis* and less than 50% of those of *Poa annua* and *P. trivialis* were recovered from the casts. Survival of seeds of other species was higher.

In a second experiment we studied the germination of seeds recovered from casts. The results show that some seeds can pass through the gut without losing their viability and that the final percentage germination was higher for such seeds than for seeds from the original stock (Table 2).

Table 2 Percentage Germination (12 Days after Sowing) of Three Seeds after Passage of Seeds through the Gut of *L. terrestris*

Species	Percentage germination		L.S.D. ($P=0.05$)
	Original stock	After passage through gut	
<i>Poa trivialis</i>	66.2	90.5	8.60
<i>Bellis perennis</i>	83.0	96.7	8.02
<i>Trifolium repens</i> (non- hard seed)	74.7	82.0	8.67
<i>T. repens</i> (hard seed)	8.3	15.4	4.96

Results of three trials each with twenty replicates of ten seeds.

Viable seeds of many species have been recovered from worm casts by both flotation and direct germination tests.

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Intelligence and Handedness

THE data of Levy¹ have been quoted^{2,3} as evidence that people with left handed preferences have significantly lower visuo-spatial IQ than right handed people, although the verbal IQ's of the two groups does not differ. I have analysed data⁴ previously obtained and classified for other purposes, to see if they support Levy's conclusions (Table 1).

Table 1 Intelligence and Handedness: Comparison of Data from Two Sources^{1,4}

	Graduate science students ¹		Academic scientists ⁴	
Numbers (handed- ness)	10 (L)	15 (R)	13 (L)	132 (R)
Verbal IQ	142 (L)	138 (R)	127.4 (L) (S.E. = 2.65)	128.2 (R) (S.E. = 0.57)
Visuo-spatial IQ	117 (L)	130 (R)	120.4 (L) (S.E. = 2.61)	120.7 (R) (S.E. = 0.78)

* Standard error.

Both studies used the Wechsler Adult Intelligence Scale⁵.

My sample of left handed people is small but provides no evidence that there is any difference in IQ components between left and right handed people.

Neither of these investigations is on an adequate scale and both are on specialized samples. It therefore seems premature to reach any conclusions about relationships between IQ or IQ components and handedness.

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BOOK REVIEWS

Space Science

Space Physics and Space Astronomy. By Michael D. Papagiannis. Pp. xiv + 293. (Gordon and Breach Science: New York, Paris and London, 1972.) £7.70.

LATE in 1957 the Russians astonished the world by creating the first artificial satellite of our Earth and by following it almost immediately by the incarceration of a dog in an orbiting death cell large enough for the world to view. Everyone will remember the successive disappointments suffered at that time by the Vanguard project and, compared with the weight of the first three Sputniks, the pathetic little Explorer I launched at last on January 31, 1958. Yet this tiny satellite with its Geiger counter payload rather than the living passenger of Sputnik II provided both a pointer to the main road to be taken by space science and the most important single discovery of the space age. By its unexpected detection of the trapped radiation, to which Singer and Christophilos had been giving theoretical consideration, this payload started the satellite study of the Earth's environment and its relationship to the Sun which has been the first great contribution of spacecraft techniques to our understanding of our world. Cosmic X-ray astronomy grew out of solar X-ray studies which were themselves part of the field of solar-terrestrial relations. To this branch of astronomy I would assign the next place in the great achievements of space science. None of this is to underrate in any way the important science coming out of manned and unmanned lunar expeditions and the instrumented flights to Venus and Mars. These, however, have not had the tremendous integrating effect which solar-terrestrial studies have had on our picture of the Earth's atmosphere, ionosphere, magnetosphere and polar phenomena in relation to the Sun, its activity, radiation, wind and high energy particle emissions. Nor do they appear to have the potential significance of our introduction to new, energetic and sometimes transient celestial phenomena through the X-ray window on the Universe. The unexpected array of cosmologically sig-

nificant phenomena from black holes to intergalactic matter and from supernova remnants to interacting binaries is comparable only to the prospects opened a decade or so earlier by the sister branch of radio astronomy.

Merely to mention radio astronomy, X-ray astronomy and so on is to remind oneself that space astronomy, space physics and indeed the whole of space science are not disciplines of science but branches of scientific disciplines marked out by the highly sophisticated technology involved. *Space Physics and Space Astronomy* sets out to introduce the graduate considering a career in space science or the professional scientist interested in it to the field as a whole. In doing so it, perhaps of necessity, largely ignores the technological basis and often even the experimental basis of the subject and reviews with readability and skill the basic geophysics, solar physics, planetary physics and astrophysics which have been the hunting ground of space research. In spite of some charming infelicities of expression betraying perhaps the Greek background of the author and some less excusable faults in proof reading the book remains one of the best introductions to the subject I have seen. Papagiannis is, himself, a notable worker in many of the areas he describes so well. Personally I could wish for a little more discussion of the experimental procedures which entitle the subject to the adjective *space* and it is noticeable that the mathematical analyses cluster round the author's own areas. Some errors of fact are obviously mistakes of expression, but I wonder if anyone still believes Cygnus-A to be a colliding system. *Space Research* is a book intended for engineers. It would have been far better to use the translator's effort in providing Papagiannis' book for Russian engineers. Part 1 is so full of errors as to be useless, even if the engineers did need to know so much geophysics and astrophysics to design spacecraft. Part 2 is concerned with the effects of space environment on materials. Like Part 1, on the whole it lists empirical data without engendering understanding. No doubt this matters less in designer's reference material but there is the somewhat unfortunate disclaimer on page 3 "The data presented

here should not be used as a standard, and they will as a rule only give rough evaluations".

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Biochemistry of Ataxia

The Pharmacology of Extrapyrarnidal Movement Disorders. By H. I. Klawans, Jr. Pp. vi + 137. (S. Karger: Basel, London and New York, 1973.) 49 Sw. francs; £5.75; \$13.75.

BASIC biochemical and pharmacological discoveries led to the prediction that levodopa would help patients with Parkinson's disease, a prediction that has been amply fulfilled in clinical practice. A side-effect of levodopa treatment is the production of abnormal movements reminiscent of those seen in diseases characterized by chorea and torsion dystonia. Naturally clinicians and basic scientists have seized on this observation as a clue to the pathogenesis of such disorders and in recent years many studies on the clinical and animal pharmacology of extrapyramidal movement disorders have been published. Dr Klawans's monograph is timely, for it draws together much of the presently available information on this topic.

The central theme of the book is the disorder of dopaminergic and cholinergic mechanisms that underlies Parkinson's disease. This is described at length from the viewpoint of a clinician who starts with observations in the clinic, and then attempts to explain them on the basis of what is known about dopamine and acetylcholine in the brain. The reasons why levodopa works (or doesn't in some patients) and the causes of the side-effects it produces are discussed at length. The value and mechanism of action of other antiparkinsonian agents are also reviewed, as is the value of levodopa in other akinetic-rigid syndromes, such as olivo-ponto-cerebellar degeneration, progressive supranuclear palsy, manganese poisoning and Wilson's disease.

Dr Klawans then turns to abnormal movement disorders such as the tardive dyskinesias produced by neuroleptics, Huntington's chorea and other choreiform states, and torsion dystonia. Building on the framework of the pharmaco-

logy of parkinsonism, he suggests that some of these abnormal movement disorders are due to over-stimulation of some dopaminergic receptors in the corpus striatum. Inevitably, much of the section is hypothetical, but the clinical, pharmacological and other evidence is marshalled in a convincing way to support the author's tentative conclusions. The hypotheses that Dr Klawans, and many other workers in the field, have put forward to explain abnormal movement disorders on a biochemical basis provide a framework for further research. Time will tell whether they are true or not, but meanwhile Dr Klawans's monograph provides a useful statement of the present evidence and will be of value to the many clinical and basic research workers interested in this expanding field of neurology.

C. D. MARSDEN

Solid State Theory

Theoretical Solid State Physics. By Albert Haug. Vol. 1. Pp. xiv+497. (Pergamon: Oxford and New York, May 1972.) £7.

Theoretical Solid State Physics. By Albert Haug. Translated by H. S. H. Massey. Edited by D. ter Haar. Vol. 2. Pp. xiii+355. (Pergamon: Oxford and New York, August 1972.) £6.

THESE volumes are translations from the German of the original editions, of which volume 1 was published in Vienna in 1964 and volume 2 in 1970. The level of treatment is suitable for a student who has completed a first degree in physics. It was the aim of the author "to make the derivations so detailed that they could be understood without any additional calculations". This aim, it must be said, is on the whole achieved. Formal mathematical derivations are set out at length, to the extent that the summation of a geometric series may be broken down into several steps. One can only presume that it was for this reason the book was thought to be sufficiently different from existing texts to merit translation. The market is, after all, well supplied with introductions to solid state theory.

The intended scope of the volumes is perhaps indicated by a quotation from the foreword to volume 2: "We are thus dealing with a closed body of solid state physics which will not be rendered superfluous by yet newer developments, and this state is represented by the present work". Volume 1 covers the elementary theory of electrons in a perfect lattice, including Wannier functions but with only a sketch of methods of band-structure calculation, the Bohm and Pines theory of the interacting electron gas, simple treatments of the magnetic and dielectric properties of solids, and the elemen-

tary theory of lattice vibrations. There is some emphasis on one-dimensional models. Volume 2 treats the electron-lattice interaction, with an account of Bardeen's classic 1937 calculation, gives a rather formal account of transport phenomena based on the Boltzmann equation, and concludes with sections on semiconductor theory and luminescence.

Even in 1964, however, volume 1 might have been thought a little out of touch. (The only updating I can find is a footnote on page 177.) Volume 2 virtually ignores the insights and results that have been gained in the past decade. A student studying these volumes in isolation would gain the impression that it is almost impossible to make calculations in solid state physics with more than order of magnitude accuracy. It was, to take one example, already clear in 1964 that the concept of a screened model potential was a valuable aid in understanding the properties of simple metals, and making realistic calculations. The Bohm and Pines theory, and the Bardeen theory of the electron-ion interaction, are landmarks in the development of solid state theory, but the equivalent dielectric constant formalism is at least as easy to present, and has proved far more convenient in practice.

It is impossible to recommend these volumes as an introduction to the current literature of the solid state. They have, perhaps, some value as a reference work for students who have local difficulties in the mathematical derivations of elementary solid state theory.

D. A. GREENWOOD

Geochemistry

Geochemical Exploration 1972. Edited by M. J. Jones. (Proceedings of the Fourth International Geochemical Exploration Symposium held in London April 17-20, 1972. Organized by the Institution of Mining and Metallurgy. Pp. xiv+458. (The Institution of Mining and Metallurgy: London, February 1973.) £12; \$30.

THIS volume contains thirty-seven papers read at the Fourth International Geochemical Exploration Symposium held in London in April 1972. They are the work of forty-four authors from twelve nations and encompass a great number of the more important aspects of the subject, though, whilst including some short case histories, they are mainly concerned with the results of research into new and developing fields. The publishers are to be congratulated on the speed with which they have produced this volume. Apart from a few over-reduced and unreadable figures, the reproduction and legibility of figures both black-and-white and coloured are of a high order.

The volume demonstrates the rapid advances being made in applied geochemistry and includes a number of "firsts". One is a paper describing anomalous metal concentrations in snow overlying ground containing sulphide ore. Another is a novel account of geological mapping and geochemical sampling using light aircraft. The methods of scooping up rock and sediment samples during flight at only a metre or so above ground level are enough to make the reader's hair stand on end. Though not quite a "first", the paper by Dr Allan and others on the use of lake sediments in reconnaissance exploration breaks fresh ground in sampling techniques. Other papers concerned with sampling in the more usual media of soil, stream sediment and rock samples cover such diverse topics as the use of gold as a pathfinder element for porphyry copper deposits, trace element patterns as a guide to ore-bearing granites, sulphur and nickel concentrations in serpentinites as a guide to mineralized areas and the use of bedrock geochemistry in regional surveys.

A landmark in the study of secondary dispersion is Professor Govett's paper in which he describes laboratory tank experiments to support the view that significant differential secondary rearrangement of metals in soils and rocks related to sulphide ore bodies may occur under the influence of electrical potentials. This could lead to new approaches to exploration through various electrophysical and chemical measurements which would allow a deeper penetration of thick transported overburden—a topic dealt with in other papers. Statistical analysis of geochemical data is given prominence in six papers and is applied in many others. Cluster analysis, pattern recognition and automated colour-mapping are included.

Mercury as a pathfinder element has attracted much interest in recent years, and it is interesting to read that studies of mercury in rock samples from an Irish deposit indicate that mercuric sulphide may be a better guide to ore than total mercury values. The use of gases is described in two contributions from the USSR which *inter alia* demonstrate their value in mapping fracture zones.

About one third of the papers deal with new techniques and instrumentation. These include the measurement of atmospheric mercury with a Zeeman spectrometer, a microwave-induced argon plasma emission system for trace analysis, fission track radiography, neutron activation analysis, *in situ* measurement of Eh and pH in diamond drill holes and the use of the ion-sensitive fluoride electrode.

A. M. EVANS